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(54) Title: CYCLIC AMP-SPECIFIC PHOSPHODIESTERASE INHIBITORS

(I)

(II)
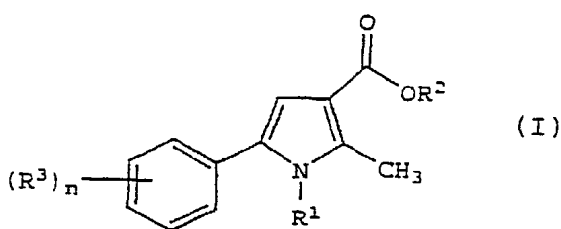
(57) Abstract: Pyrrole compounds having structural formula (I) or (II) that are potent and selective inhibitors of PDE4, as well as methods of making the same, are disclosed. Use of the compounds in the treatment of inflammatory diseases and other diseases involving elevated levels of cytokines, as well as central nervous system (CNS) disorders, also is disclosed.

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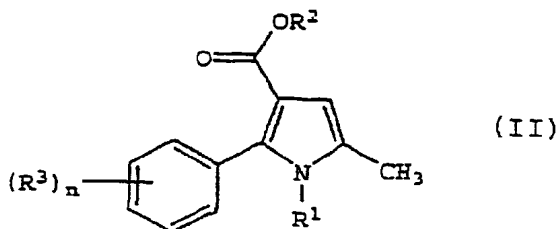
CYCLIC AMP-SPECIFIC PHOSPHODIESTERASE INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATION

5   This application claims the benefit of
provisional application Serial No. 60/171,954, filed
December 23, 1999.

FIELD OF INVENTION

10
           The present invention relates to a series
of compounds that are potent and selective inhibi-
tors of cyclic adenosine 3',5'-monophosphate spe-
cific phosphodiesterase (cAMP-specific PDE). In
15   particular, the present invention relates to a se-
ries of novel pyrrole compounds that are useful for
inhibiting the function of cAMP-specific PDE, in
particular, PDE4, as well as methods of making the
same, pharmaceutical compositions containing the
20   same, and their use as therapeutic agents, for
example, in treating inflammatory diseases and other
diseases involving elevated levels of cytokines and
proinflammatory mediators.

25   BACKGROUND OF THE INVENTION

           Chronic inflammation is a multi-factorial
disease complication characterized by activation of
multiple types of inflammatory cells, particularly
30   cells of lymphoid lineage (including T lymphocytes)
and myeloid lineage (including granulocytes, macro-
phages, and monocytes). Proinflammatory mediators,
including cytokines, such as tumor necrosis factor

- 2 -

(TNF) and interleukin-1 (IL-1), are produced by
these activated cells. Accordingly, an agent that
suppresses the activation of these cells, or their
production of proinflammatory cytokines, would be
5   useful in the therapeutic treatment of inflammatory
diseases and other diseases involving elevated lev-
els of cytokines.

Cyclic adenosine monophosphate (cAMP) is a
second messenger that mediates the biologic re-
10   sponses of cells to a wide range of extracellular
stimuli. When the appropriate agonist binds to
specific cell surface receptors, adenylate cyclase
is activated to convert adenosine triphosphate (ATP)
to cAMP. It is theorized that the agonist induced
15   actions of cAMP within the cell are mediated predom-
inately by the action of cAMP-dependent protein
kinases. The intracellular actions of cAMP are
terminated by either a transport of the nucleotide
to the outside of the cell, or by enzymatic cleavage
20   by cyclic nucleotide phosphodiesterases (PDEs),
which hydrolyze the 3'-phosphodiester bond to form
5'-adenosine monophosphate (5'-AMP). 5'-AMP is an
inactive metabolite. The structures of cAMP and 5'-
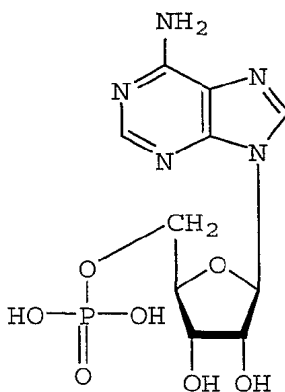AMP are illustrated below.

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- 3 -
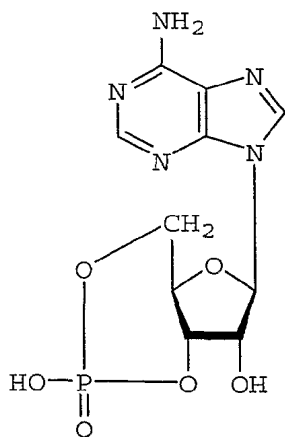
cAMP

5'-AMP

Elevated levels of cAMP in human myeloid and lymphoid lineage cells are associated with the suppression of cell activation. The intracellular enzyme family of PDEs, therefore, regulates the level of cAMP in cells. PDE4 is a predominant PDE isotype in these cells, and is a major contributor to cAMP degradation. Accordingly, the inhibition of

- 4 -

PDE function would prevent the conversion of cAMP to the inactive metabolite 5'-AMP and, consequently, maintain higher cAMP levels, and, accordingly, suppress cell activation (see Beavo et al., "Cyclic
5   Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action," Wiley and Sons, Chichester, pp. 3-14, (1990)); Torphy et al., *Drug News and Perspectives, 6*, pp. 203-214 (1993); Giembycz et al., *Clin. Exp. Allergy, 22*, pp. 337-344 (1992)).
10   In particular, PDE4 inhibitors, such as rolipram, have been shown to inhibit production of TNFα and partially inhibit IL-1β release by monocytes (see Semmler et al., *Int. J. Immunopharmacol., 15*, pp. 409-413, (1993); Molnar-Kimber et al., *Medi-*
15   *ators of Inflammation, 1*, pp. 411-417, (1992)). PDE4 inhibitors also have been shown to inhibit the production of superoxide radicals from human polymorphonuclear leukocytes (see Verghese et al., *J. Mol. Cell. Cardiol., 21 (Suppl. 2), S61* (1989);
20   Nielson et al., *J. Allergy Immunol., 86*, pp. 801-808, (1990)); to inhibit the release of vasoactive amines and prostanoids from human basophils (see Peachell et al., *J. Immunol., 148*, pp. 2503-2510, (1992)); to inhibit respiratory bursts in eosino-
25   phils (see Dent et al., *J. Pharmacol., 103*, pp. 1339-1346, (1991)); and to inhibit the activation of human T-lymphocytes (see Robicsek et al., *Biochem. Pharmacol., 42*, pp. 869-877, (1991)).
             Inflammatory cell activation and excessive
30   or unregulated cytokine (e.g., TNFα and IL-1β) production are implicated in allergic, autoimmune, and inflammatory diseases and disorders, such as rheuma-

- 5 -

toid arthritis, osteoarthritis, gouty arthritis,
spondylitis, thyroid associated ophthalmopathy,
Behcet's disease, sepsis, septic shock, endotoxic
shock, gram negative sepsis, gram positive sepsis,
5   toxic shock syndrome, asthma, chronic bronchitis,
adult respiratory distress syndrome, chronic pulmo-
nary inflammatory disease, such as chronic obstruc-
tive pulmonary disease, silicosis, pulmonary sarco-
idosis, reperfusion injury of the myocardium, brain,
10   and extremities, fibrosis, cystic fibrosis, keloid
formation, scar formation, atherosclerosis, trans-
plant rejection disorders, such as graft vs. host
reaction and allograft rejection, chronic glomerulo-
nephritis, lupus, inflammatory bowel disease, such
15   as Crohn's disease and ulcerative colitis, prolif-
erative lymphocyte diseases, such as leukemia, and
inflammatory dermatoses, such as atopic dermatitis,
psoriasis, and urticaria.

        Other conditions characterized by elevated
20   cytokine levels include brain injury due to moderate
trauma (see Dhillon et al., *J. Neurotrauma*, *12*, pp.
1035-1043 (1995); Suttorp et al., *J. Clin. Invest.*,
91, pp. 1421-1428 (1993)), cardiomyopathies, such as
congestive heart failure (see Bristow et al., *Circu-*
25   *lation*, *97*, pp. 1340-1341 (1998)), cachexia, cachex-
ia secondary to infection or malignancy, cachexia
secondary to acquired immune deficiency syndrome
(AIDS), ARC (AIDS related complex), fever myalgias
due to infection, cerebral malaria, osteoporosis and
30   bone resorption diseases, keloid formation, scar
tissue formation, and pyrexia.

        In particular, TNFα has been identified as
having a role with respect to human acquired immune

- 6 -

deficiency syndrome (AIDS). AIDS results from the
infection of T-lymphocytes with Human Immunodefi-
ciency Virus (HIV). Although HIV also infects and
is maintained in myeloid lineage cells, TNF has been
5   shown to upregulate HIV infection in T-lymphocytic
and monocytic cells (see Poli et al., *Proc. Natl.
Acad. Sci. USA, 87*, pp. 782-785, (1990)).

Several properties of TNFα, such as stimu-
lation of collagenases, stimulation of angiogenesis
10   *in vivo*, stimulation of bone resorption, and an
ability to increase the adherence of tumor cells to
endothelium, are consistent with a role for TNF in
the development and metastatic spread of cancer in
the host. TNFα recently has been directly impli-
15   cated in the promotion of growth and metastasis of
tumor cells (see Orosz et al., *J. Exp. Med., 177*,
pp. 1391-1398, (1993)).

PDE4 has a wide tissue distribution.
There are at least four genes for PDE4 of which
20   multiple transcripts from any given gene can yield
several different proteins that share identical
catalytic sites. The amino acid identity between
the four possible catalytic sites is greater than
85%. Their shared sensitivity to inhibitors and
25   their kinetic similarity reflect the functional
aspect of this level of amino acid identity. It is
theorized that the role of these alternatively
expressed PDE4 proteins allows a mechanism by which
a cell can differentially localize these enzymes
30   intracellularly and/or regulate the catalytic effi-
ciency *via* post translational modification. Any
given cell type that expresses the PDE4 enzyme typi-

- 7 -

cally expresses more than one of the four possible
genes encoding these proteins.

Investigators have shown considerable
interest in the use of PDE4 inhibitors as anti-in-
5   flammatory agents. Early evidence indicates that
PDE4 inhibition has beneficial effects on a variety
of inflammatory cells such as monocytes, macro-
phages, T-cells of the Th-1 lineage, and granulo-
cytes. The synthesis and/or release of many pro-
10   inflammatory mediators, such as cytokines, lipid
mediators, superoxide, and biogenic amines, such as
histamine, have been attenuated in these cells by
the action of PDE4 inhibitors. The PDE4 inhibitors
also affect other cellular functions including T-
15   cell proliferation, granulocyte transmigration in
response to chemotoxic substances, and integrity of
endothelial cell junctions within the vasculature.

The design, synthesis, and screening of
various PDE4 inhibitors have been reported. Methyl-
20   xanthines, such as caffeine and theophylline, were
the first PDE inhibitors discovered, but these com-
pounds are nonselective with respect to which PDE is
inhibited. The drug rolipram, an antidepressant
agent, was one of the first reported specific PDE4
25   inhibitors. Rolipram, having the following struc-
tural formula, has a reported 50% Inhibitory Concen-
tration (IC_{50}) of about 200 nM (nanomolar) with re-
spect to inhibiting recombinant human PDE4.

- 8 -
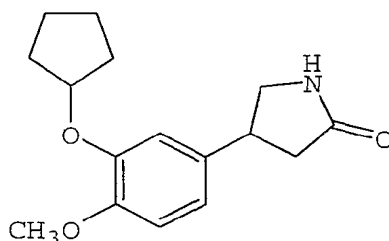
Rolipram

Investigators have continued to search for PDE4 inhibitors that are more selective with respect to inhibiting PDE4, that have a lower IC_{50} than rolipram, and that avoid the undesirable central nervous system (CNS) side effects, such as retching, vomiting, and sedation, associated with the administration of rolipram. One class of compounds is disclosed in Feldman et al. U.S. Patent No. 5,665,754. The compounds disclosed therein are substituted pyrrolidines having a structure similar to rolipram. One particular compound, having the following structural formula, has an IC_{50} with respect to human recombinant PDE4 of about 2 nM. Inasmuch as a favorable separation of emetic side effect from efficacy was observed, these compounds did not exhibit a reduction in undesirable CNS effects.
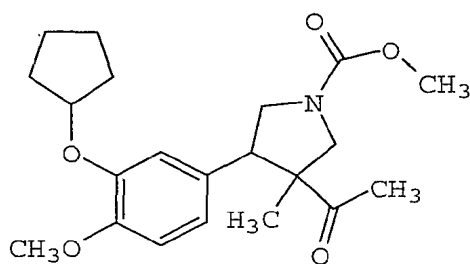
- 9 -

In addition, several companies are now undertaking clinical trials of other PDE4 inhibitors. However, problems relating to efficacy and adverse side effects, such as emesis and central
5   nervous system disturbances, remain unsolved.

Accordingly, compounds that selectively inhibit PDE4, and that reduce or eliminate the adverse CNS side effects associated with prior PDE4 inhibitors, would be useful in the treatment of
10   allergic and inflammatory diseases, and other diseases associated with excessive or unregulated production of cytokines, such as TNF. In addition, selective PDE4 inhibitors would be useful in the treatment of diseases that are associated with ele-
15   vated cAMP levels or PDE4 function in a particular target tissue.

SUMMARY OF THE INVENTION

20   The present invention is directed to potent and selective PDE4 inhibitors useful in treatment of diseases and conditions where inhibition of PDE4 activity is considered beneficial. The present PDE4 inhibitors unexpectedly reduce or eliminate the
25   adverse CNS side effects associated with prior PDE4 inhibitors.

In particular, the present invention is directed to pyrrole compounds having the structural formula (I) or (II):
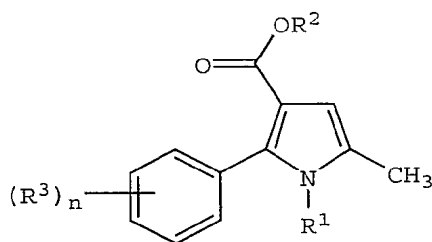
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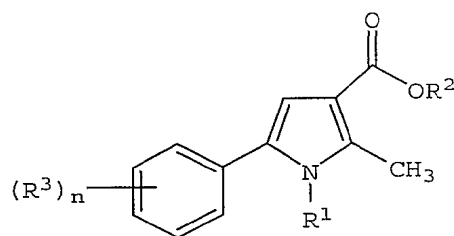
(I)

10

15

(II)

wherein R^1 is selected from the group con-
20   sisting of optionally substituted aryl, alkyl,
cycloalkyl, heteroaryl, alkaryl, aralkyl, hetero-
aralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of
25   alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, hetero-
aryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)$alkyl, NR^4R^5,
$C(=O)NR^5R^6$, $C(=O)O$alkyl, CO_2H, $OC(=O)$alkyl, nitro,
halo, alkylthio, SO_2(alkyl), SO_3H, and haloalkyl;

R^4 and R^5, independently, are hydrogen or
30   alkyl, or R^5 or R^6 are taken together to form a 5- or
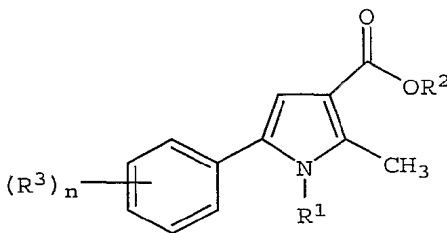6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3.

- 11 -

The present invention also is directed to pharmaceutical compositions containing one or more of the compounds of structural formula (I) or (II), to use of the compounds and compositions containing
5   the compounds in the treatment of a disease or disorder, and to methods of preparing the compounds and intermediates involved in the synthesis of the compounds of structural formula (I) and (II).

10   **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 contains plots of TNFα concentration in serum (pg/mL) vs. concentration of PDE4 inhibitors.
15

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS
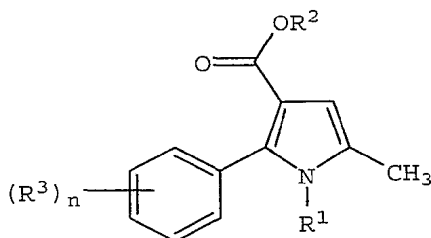
The present invention is directed to compounds having a structural formula (I) or (II):
20

25

(I)

30

- 12 -

$$O = C \begin{matrix} OR^2 \end{matrix}$$

(R^3)_n —[phenyl]— [pyrrole ring] — CH_3

N
R^1

(II)

wherein

R^1 is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, hetero-aryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)$alkyl, NR^4R^5, $C(=O)NR^5R^6$, $C(=O)O$alkyl, CO_2H, $OC(=O)$alkyl, nitro, halo, alkylthio, SO_2(alkyl), SO_3H, and haloalkyl;

R^4 and R^5, independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3.

As used herein, the term "alkyl," alone or in combination, is defined to include straight chain and branched chain, and bridged, saturated hydrocar-bon groups containing one to 16 carbon atoms. The term "lower alkyl" is defined herein as an alkyl group having one through six carbon atoms (C_1-C_6). Examples of lower alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, iso-butyl, n-butyl, neopentyl, -hexyl, and the like.

- 13 -

The term "bridged alkyl" is defined herein as a C_6-C_{16} bicyclic or polycyclic hydrocarbon group, for example, norboryl, adamantyl, bicyclo[2.2.2]-octyl, bicyclo[2.2.1]heptyl, bicyclo[3.2.1]octyl, or decahydronaphthyl.

The term "cycloalkyl" is defined herein to include cyclic C_3-C_7 hydrocarbon groups. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclohexyl, and cyclo-pentyl.

The term "haloalkyl" is defined herein as an alkyl group substituted with one or more halo substituents, either fluoro, chloro, bromo, iodo, or combinations thereof. Similarly, "halocycloalkyl" is defined as a cycloalkyl group having one or more halo substituents.

The term "aryl," alone or in combination, is defined herein as a monocyclic or polycyclic aromatic group, preferably a monocyclic or bicyclic aromatic group, e.g., phenyl or naphthyl, that can be unsubstituted or substituted, for example, with one or more, and in particular one to three, sub-stituents selected from halo, alkyl, hydroxy, hy-droxyalkyl, alkoxy, alkoxyalkyl, haloalkyl, nitro, amino, alkylamino, acylamino, alkylthio, alkyl-sulfinyl, and alkylsulfonyl. Exemplary aryl groups include phenyl, naphthyl, tetrahydronaphthyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 2-methylphenyl, 4-methoxyphenyl, 3-trifluoromethyl-phenyl, 4-nitrophenyl, and the like.

The term "heteroaryl" is defined herein as a monocyclic or bicyclic ring system containing one or two aromatic rings and containing at least one

- 14 -

nitrogen, oxygen, or sulfur atom in an aromatic
ring, and which can be unsubstituted or substituted,
for example, with one or more, and in particular one
to three, substituents, like halo, alkyl, hydroxy,
5   hydroxyalkyl, alkoxy, alkoxyalkyl, haloalkyl, nitro,
amino, alkylamino, acylamino, alkylthio, alkylsul-
finyl, and alkylsulfonyl. Examples of heteroaryl
groups include thienyl, furyl, pyridyl, oxazolyl,
quinolyl, isoquinolyl, indolyl, triazolyl, isothi-
10   azolyl, isoxazolyl, imidizolyl, benzothiazolyl,
pyrazinyl, pyrimidinyl, thiazolyl, and thiadiazolyl.
        The term "aralkyl" is defined herein as a
previously defined alkyl group, wherein one of the
hydrogen atoms is replaced by an aryl group as de-
15   fined herein, for example, a phenyl group optionally
having one or more substituents, for example, halo,
alkyl, alkoxy, and the like. An example of an
aralkyl group is a benzyl group.
        The term "alkaryl" is defined herein as a
20   previously defined aryl group, wherein one of the
hydrogen atoms is replaced by an alkyl, cycloalkyl,
haloalkyl, or halocycloalkyl group.
        The term "heteroaralkyl" and "hetero-
alkaryl" are defined similarly as the terms
25   "aralkyl" and "alkaryl," however, the aryl group is
replaced by a heteroaryl group as previously de-
fined.
        The term "heterocycle" or "heterocyclic
ring" is defined as a 5- or 6-membered nonaromatic
30   ring having one or more heteroatoms selected from
oxygen, nitrogen, and sulfur present in the ring.
Nonlimiting examples include tetrahydrofuran, piper-
idine, piperazine, sulfolane, morpholine, tetra-

- 15 -

hydropyran, dioxane, and the like. A "carbocyclic ring" is similarly defined, but the ring contains solely carbon atoms.

The term "halogen" or "halo" is defined
5   herein to include fluorine, chlorine, bromine, and iodine.

The terms "alkoxy," "aryloxy," and "aralkoxy" are defined as -OR, wherein R is alkyl, aryl, and aralkyl, respectively.

10   The term "alkoxyalkyl" is defined as an alkoxy group appended to an alkyl group. The terms "aryloxyalkyl" and "aralkoxyalkyl" are similarly defined as an aryloxy or aralkoxy group appended to an alkyl group.

15   The term "hydroxy" is defined as -OH.

The term "hydroxyalkyl" is defined as a hydroxy group appended to an alkyl group.

The term "amino" is defined as $-NH_2$.

The term "alkylamino" is defined as $-NR_2$
20   wherein at least one R is alkyl and the second R is alkyl or hydrogen.

The term "acylamino" is defined as RC(=O)NH, wherein R is alkyl or aryl.

The term "nitro" is defined as $-NO_2$.
25   The term "alkylthio" is defined as -SR, where R is alkyl.

The term "alkylsulfinyl" is defined as $R-S(O)_2$, where R is alkyl.

The term "alkylsulfonyl" is defined as
30    $R-S(O_3)$, where R is alkyl.

In preferred embodiments, R^1 is selected from the group consisting of aryl, heteroaryl,

- 16 -

alkaryl, aralkyl, and cycloalkyl; R^2 is selected from the group consisting of hydrogen and lower alkyl.

 In most preferred embodiments, R^1 is aryl, heteroaryl, cycloalkyl, or aralkyl, optionally sub-

5 stituted with one or more of NR^4R^5, halo, alkyl, $C(=O)$alkyl, SO_2(alkyl), $C(=O)NR^4R^5$, SO_3H, NO_2, CO_2H, $C(=O)O$alkyl, and trifluoromethyl; R^2 is hydrogen or C_{1-2}alkyl, and R^3 is hydrogen. R^1 typically is phenyl or pyridyl, optionally substituted, and R^2 is hydro-

10 gen or ethyl.

 The present invention includes all possible stereoisomers and geometric isomers of compounds of structural formulae (I) and (II), and includes not only racemic compounds but also the optically

15 active isomers as well. When a compound of structural formulae (I) and (II) is desired as a single enantiomer, it can be obtained either by resolution of the final product or by stereospecific synthesis from either isomerically pure starting material or

20 use of a chiral auxiliary reagent, for example, see Z. Ma et al., *Tetrahedron: Asymmetry*, 8(6), pages 883-888 (1997). Resolution of the final product, an intermediate, or a starting material can be achieved by any suitable method known in the art. Addition-

25 ally, in situations where tautomers of the compounds of structural formulae (I) and (II) are possible, the present invention is intended to include all tautomeric forms of the compounds. As demonstrated hereafter, specific stereoisomers exhibit an excep-

30 tional ability to inhibit PDE4 without manifesting the adverse CNS side effects typically associated with PDE4 inhibitors.

- 17 -

Compounds of structural formula (I) and
(II) which contain acidic moieties can form pharma-
ceutically acceptable salts with suitable cations.
Suitable pharmaceutically acceptable cations include
5   alkali metal (e.g., sodium or potassium) and alka-
line earth metal (e.g., calcium or magnesium) cat-
ions. The pharmaceutically acceptable salts of the
compounds of structural formula (I) and (II), which
contain a basic center, are acid addition salts
10   formed with pharmaceutically acceptable acids.
Examples include the hydrochloride, hydrobromide,
sulfate or bisulfate, phosphate or hydrogen phos-
phate, acetate, benzoate, succinate, fumarate, male-
ate, lactate, citrate, tartrate, gluconate, methane-
15   sulfonate, benzenesulphonate, and p-toluenesul-
phonate salts. In light of the foregoing, any ref-
erence to compounds of the present invention appear-
ing herein is intended to include compounds of
structural formula (I) and (II), as well as pharma-
20   ceutically acceptable salts and solvates thereof.

The compounds of the present invention can
be therapeutically administered as the neat chemi-
cal, but it is preferable to administer compounds of
structural formula (I) and (II) as a pharmaceutical
25   composition or formulation. Accordingly, the pres-
ent invention further provides for pharmaceutical
formulations comprising a compound of structural
formula (I) and/or (II), or pharmaceutically accept-
able salts thereof, together with one or more phar-
30   maceutically acceptable carriers and, optionally,
other therapeutic and/or prophylactic ingredients.
The carriers are "acceptable" in the sense of being

- 18 -

compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

In particular, a selective PDE4 inhibitor of the present invention is useful alone or in combination with a second antiinflammatory therapeutic agent, for example, a therapeutic agent targeting TNFα, such as ENBREL® or REMICADE®, which have utility in treating rheumatoid arthritis. Likewise, therapeutic utility of IL-1 antagonism has also been shown in animal models for rheumatoid arthritis. Thus, it is envisioned that IL-1 antagonism, in combination with PDE4 inhibition, which attenuates TNFα, would be efficacious.

The present PDE4 inhibitors are useful in the treatment of a variety of allergic, autoimmune, and inflammatory diseases.

The term "treatment" includes preventing, lowering, stopping, or reversing the progression of severity of the condition or symptoms being treated. As such, the term "treatment" includes both medical therapeutic and/or prophylactic administration, as appropriate.

In particular, inflammation is a localized, protective response elicited by injury or destruction of tissues, which serves to destroy, dilute or wall off (i.e., sequester) both the injurious agent and the injured tissue. The term "inflammatory disease," as used herein, means any disease in which an excessive or unregulated inflammatory response leads to excessive inflammatory symptoms, host tissue damage, or loss of tissue function. Additionally, the term "autoimmune disease," as used herein, means any group of disorders in

which tissue injury is associated with humoral or cell-mediated responses to the body's own constituents. The term "allergic disease," as used herein, means any symptoms, tissue damage, or loss of tissue

5   function resulting from allergy. The term "arthritic disease," as used herein, means any of a large family of diseases that are characterized by inflammatory lesions of the joints attributable to a variety of etiologies. The term "dermatitis," as used

10   herein, means any of a large family of diseases of the skin that are characterized by inflammation of the skin attributable to a variety of etiologies. The term "transplant rejection," as used herein, means any immune reaction directed against grafted

15   tissue (including organ and cell (e.g., bone marrow)), characterized by a loss of function of the grafted and surrounding tissues, pain, swelling, leukocytosis and thrombocytopenia.

         The present invention also provides a

20   method of modulating cAMP levels in a mammal, as well as a method of treating diseases characterized by elevated cytokine levels.

         The term "cytokine," as used herein, means any secreted polypeptide that affects the functions

25   of other cells, and that modulates interactions between cells in the immune or inflammatory response. Cytokines include, but are not limited to monokines, lymphokines, and chemokines regardless of which cells produce them. For instance, a monokine

30   is generally referred to as being produced and secreted by a monocyte, however, many other cells produce monokines, such as natural killer cells, fibroblasts, basophils, neutrophils, endothelial

- 20 -

cells, brain astrocytes, bone marrow stromal cells, epidermal keratinocytes, and B-lymphocytes. Lympho- kines are generally referred to as being produced by lymphocyte cells. Examples of cytokines include,

5   but are not limited to, interleukin-1 (IL-1), inter- leukin-6 (IL-6), Tumor Necrosis Factor alpha (TNFα), and Tumor Necrosis Factor beta (TNFβ).

The present invention further provides a method of reducing TNF levels in a mammal, which

10   comprises administering an effective amount of a compound of structural formula (II) to the mammal. The term "reducing TNF levels," as used herein, means either:

a)   decreasing excessive *in vivo* TNF

15   levels in a mammal to normal levels or below normal levels by inhibition of the *in vivo* release of TNF by all cells, including but not limited to monocytes or macrophages; or

b)   inducing a down-regulation, at the

20   translational or transcription level, of excessive *in vivo* TNF levels in a mammal to normal levels or below normal levels; or

c)   inducing a down-regulation, by inhi- bition of the direct synthesis of TNF as a postrans-

25   lational event.

Moreover, the compounds of the present invention are useful in suppressing inflammatory cell activation. The term "inflammatory cell acti- vation," as used herein, means the induction by a

30   stimulus (including, but not limited to, cytokines, antigens or auto-antibodies) of a proliferative cellular response, the production of soluble media- tors (including but not limited to cytokines, oxygen

- 21 -

radicals, enzymes, prostanoids, or vasoactive amines), or cell surface expression of new or increased numbers of mediators (including, but not limited to, major histocompatability antigens or

5   cell adhesion molecules) in inflammatory cells (including but not limited to monocytes, macrophages, T lymphocytes, B lymphocytes, granulocytes, polymorphonuclear leukocytes, mast cells, basophils, eosinophils, dendritic cells, and endothelial

10   cells). It will be appreciated by persons skilled in the art that the activation of one or a combination of these phenotypes in these cells can contribute to the initiation, perpetuation, or exacerbation of an inflammatory condition.

15   The compounds of the present invention also are useful in causing airway smooth muscle relaxation, bronchodilation, and prevention of bronchoconstriction.

            The compounds of the present invention,

20   therefore, are useful in treating such diseases as arthritic diseases (such as rheumatoid arthritis), osteoarthritis, gouty arthritis, spondylitis, thyroid-associated ophthalmopathy, Behcet disease, sepsis, septic shock, endotoxic shock, gram negative

25   sepsis, gram positive sepsis, toxic shock syndrome, asthma, chronic bronchitis, allergic rhinitis, allergic conjunctivitis, vernal conjunctivitis, eosinophilic granuloma, adult (acute) respiratory distress syndrome (ARDS), chronic pulmonary inflam-

30   matory disease (such as chronic obstructive pulmonary disease), silicosis, pulmonary sarcoidosis, reperfusion injury of the myocardium, brain or extremities, brain or spinal cord injury due to minor

- 22 -

trauma, fibrosis including cystic fibrosis, keloid formation, scar tissue formation, atherosclerosis, autoimmune diseases, such as systemic lupus erythematosus (SLE) and transplant rejection disor-
5   ders (e.g., graft vs. host (GvH) reaction and allograft rejection), chronic glomerulonephritis, inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, proliferative lymphocytic diseases, such as leukemias (e.g. chronic lympho-
10 cytic leukemia; CLL) (see Mentz et al., *Blood 88*, pp. 2172-2182 (1996)), and inflammatory dermatoses, such as atopic dermatitis, psoriasis, or urticaria.

Other examples of such diseases or related conditions include cardiomyopathies, such as conges-
15 tive heart failure, pyrexia, cachexia, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), ARC (AIDS-related complex), cerebral malaria, osteoporosis and bone resorption diseases,
20 and fever and myalgias due to infection. In addition, the compounds of the present invention are useful in the treatment of diabetes insipidus and central nervous system disorders, such as depression and multiinfarct dementia.

25 Compounds of the present invention also have utility outside of that typically known as therapeutic. For example, the present compounds can function as organ transplant preservatives (see Pinsky et al., *J. Clin. Invest.*, 92, pp. 2994-3002
30 (1993)) as well.

Selective PDE4 inhibitors also can be useful in the treatment of diabetes insipidus (*Kidney Int.*, 37, p. 362, (1990); *Kidney Int.*, 35, p.

- 23 -

494, (1989)) and central nervous system disorders, such as multiinfarct dementia (Nicholson, *Psychopharmacology, 101*, p. 147 (1990)), depression (Eckman et al., *Curr. Ther. Res., 43*, p. 291

5   (1988)), anxiety and stress responses (*Neuropharmacology, 38*, p. 1831 (1991)), cerebral ischemia (*Eur. J. Pharmacol., 272*, p. 107 (1995)), tardive dyskinesia (*J. Clin. Pharmocol., 16*, p. 304 (1976)), Parkinson's disease (see *Neurology, 25*, p. 722

10   (1975); *Clin. Exp. Pharmacol, Physiol., 26*, p. 421 (1999)), and premenstrual syndrome. With respect to depression, PDE4-selective inhibitors show efficacy in a variety of animal models of depression such as the "behavioral despair" or Porsolt tests (*Eur. J.*

15   *Pharmacol., 47*, p. 379 (1978); *Eur. J. Pharmacol., 57*, p. 431 (1979); *Antidepressants: neurochemical, behavioral and clinical prospectives*, Enna, Malick, and Richelson, eds., Raven Press, p. 121 (1981)), and the "tail suspension test" (*Psychopharmacology,*

20   *85*, p. 367 (1985)). Recent research findings show that chronic *in vivo* treatment by a variety of antidepressants increase the brain-derived expression of PDE4 (*J. Neuroscience, 19*, p. 610 (1999)). Therefore, a selective PDE4 inhibitor can be used alone

25   or in conjunction with a second therapeutic agent in a treatment for the four major classes of antidepressants: electroconvulsive procedures, monoamine oxidase inhibitors, and selective reuptake inhibitors of serotonin or norepinephrine. Selective PDE4

30   inhibitors also can be useful in applications that modulate bronchodilatory activity via direct action

- 24 -

on bronchial smooth muscle cells for the treatment
of asthma.

Compounds and pharmaceutical compositions
suitable for use in the present invention include
5   those wherein the active ingredient is administered
in an effective amount to achieve its intended pur-
pose. More specifically, a "therapeutically effec-
tive amount" means an amount effective to prevent
development of, or to alleviate the existing symp-
10   toms of, the subject being treated. Determination
of the effective amounts is well within the capabil-
ity of those skilled in the art, especially in light
of the detailed disclosure provided herein.

A "therapeutically effective dose" refers
15   to that amount of the compound that results in
achieving the desired effect. Toxicity and thera-
peutic efficacy of such compounds can be determined
by standard pharmaceutical procedures in cell cul-
tures or experimental animals, e.g., for determining
20   the LD_{50} (the dose lethal to 50% of the population)
and the ED_{50} (the dose therapeutically effective in
50% of the population). The dose ratio between
toxic and therapeutic effects is the therapeutic
index, which is expressed as the ratio between LD_{50}
25   and ED_{50}. Compounds which exhibit high therapeutic
indices are preferred. The data obtained from such
data can be used in formulating a dosage range for
use in humans. The dosage of such compounds prefer-
ably lies within a range of circulating concentra-
30   tions that include the ED_{50} with little or no toxic-
ity. The dosage can vary within this range depend-
ing upon the dosage form employed, and the route of
administration utilized.

- 25 -

The exact formulation, route of adminis-
tration, and dosage can be chosen by the individual
physician in view of the patient's condition. Dos-
age amount and interval can be adjusted individually
5   to provide plasma levels of the active moiety which
are sufficient to maintain the therapeutic effects.

As appreciated by persons skilled in the
art, reference herein to treatment extends to pro-
phylaxis, as well as to treatment of established
10   diseases or symptoms. It is further appreciated
that the amount of a compound of the invention re-
quired for use in treatment varies with the nature
of the condition being treated, and with the age and
the condition of the patient, and is ultimately
15   determined by the attendant physician or veterinar-
ian. In general, however, doses employed for adult
human treatment typically are in the range of 0.001
mg/kg to about 100 mg/kg per day. The desired dose
can be conveniently administered in a single dose,
20   or as multiple doses administered at appropriate
intervals, for example as two, three, four or more
subdoses per day. In practice, the physician deter-
mines the actual dosing regimen which is most suit-
able for an individual patient, and the dosage var-
25   ies with the age, weight, and response of the par-
ticular patient. The above dosages are exemplary of
the average case, but there can be individual in-
stances in which higher or lower dosages are mer-
ited, and such are within the scope of the present
30   invention.

Formulations of the present invention can
be administered in a standard manner for the treat-
ment of the indicated diseases, such as orally,

- 26 -

parenterally, transmucosally (e.g., sublingually or
via buccal administration), topically, transderm-
ally, rectally, via inhalation (e.g., nasal or deep
lung inhalation). Parenteral administration in-

5   cludes, but is not limited to intravenous, intra-
arterial, intraperitoneal, subcutaneous, intramuscu-
lar, intrathecal, and intraarticular. Parenteral
administration also can be accomplished using a high
pressure technique, like POWDERJECT™.

10   For buccal administration, the composition
can be in the form of tablets or lozenges formulated
in conventional manner. For example, tablets and
capsules for oral administration can contain conven-
tional excipients such as binding agents (for exam-

15   ple, syrup, accacia, gelatin, sorbitol, tragacanth,
mucilage of starch or polyvinylpyrrolidone), fillers
(for example, lactose, sugar, microcrystalline,
cellulose, maize-starch, calcium phosphate or sorbi-
tol), lubricants (for example, magnesium, stearate,

20   stearic acid, talc, polyethylene glycol or silica),
disintegrants (for example, potato starch or sodium
starch glycollate), or wetting agents (for example,
sodium lauryl sulfate). The tablets can be coated
according to methods well known in the art.

25   Alternatively, the compounds of the pres-
ent invention can be incorporated into oral liquid
preparations such as aqueous or oily suspensions,
solutions, emulsions, syrups, or elixirs, for exam-
ple. Moreover, formulations containing these com-

30   pounds can be presented as a dry product for consti-
tution with water or other suitable vehicle before
use. Such liquid preparations can contain conven-
tional additives, such as suspending agents, such as

- 27 -

sorbitol syrup, methyl cellulose, glucose/sugar
syrup, gelatin, hydroxyethylcellulose, hydroxy-
propylmethylcellulose, carboxymethyl cellulose,
aluminum stearate gel, and hydrogenated edible fats;

5   emulsifying agents, such as lecithin, sorbitan mono-
oleate, or acacia; nonaqueous vehicles (which can
include edible oils), such as almond oil, fraction-
ated coconut oil, oily esters, propylene glycol, and
ethyl alcohol; and preservatives, such as methyl or

10 propyl p-hydroxybenzoate and sorbic acid.

        Such preparations also can be formulated
as suppositories, e.g., containing conventional
suppository bases, such as cocoa butter or other
glycerides. Compositions for inhalation typically

15 can be provided in the form of a solution, suspen-
sion, or emulsion that can be administered as a dry
powder or in the form of an aerosol using a conven-
tional propellant, such as dichlorodifluoromethane
or trichlorofluoromethane. Typical topical and

20 transdermal formulations comprise conventional aque-
ous or nonaqueous vehicles, such as eye drops,
creams, ointments, lotions, and pastes, or are in
the form of a medicated plaster, patch, or membrane.

        Additionally, compositions of the present

25 invention can be formulated for parenteral adminis-
tration by injection or continuous infusion. Formu-
lations for injection can be in the form of suspen-
sions, solutions, or emulsions in oily or aqueous
vehicles, and can contain formulation agents, such

30 as suspending, stabilizing, and/or dispersing
agents. Alternatively, the active ingredient can be
in powder form for constitution with a suitable

- 28 -

vehicle (e.g., sterile, pyrogen-free water) before
use.

A composition in accordance with the
present invention also can be formulated as a depot
5   preparation. Such long acting formulations can be
administered by implantation (for example, subcuta-
neously or intramuscularly) or by intramuscular
injection. Accordingly, the compounds of the inven-
tion can be formulated with suitable polymeric or
10   hydrophobic materials (e.g., an emulsion in an ac-
ceptable oil), ion exchange resins, or as sparingly
soluble derivatives (e.g., a sparingly soluble
salt).

For veterinary use, a compound of formula
15   (I) or (II), or nontoxic salts thereof, is adminis-
tered as a suitably acceptable formulation in accor-
dance with normal veterinary practice. The veteri-
narian can readily determine the dosing regimen and
route of administration that is most appropriate for
20   a particular animal.

Thus, the invention provides in a further
aspect a pharmaceutical composition comprising a
compound of the formula (I) or (II), together with a
pharmaceutically acceptable diluent or carrier
25   therefor. There is further provided by the present
invention a process of preparing a pharmaceutical
composition comprising a compound of formula (I) or
(II), which process comprises mixing a compound of
formula (I) or (II), together with a pharmaceuti-
30   cally acceptable diluent or carrier therefor.

Specific, nonlimiting examples of com-
pounds of structural formula (I) and (II) are pro-

- 29 -

vided below, the synthesis of which were performed
in accordance with the procedures set forth below.

Generally, compounds of structural formula
(I) and (II) can be prepared according to the fol-

5   lowing synthetic scheme. In the scheme described
below, it is understood in the art that protecting
groups can be employed where necessary in accordance
with general principles of synthetic chemistry.
These protecting groups are removed in the final

10   steps of the synthesis under basic, acidic, or
hydrogenolytic conditions which are readily apparent
to those skilled in the art. By employing appropri-
ate manipulation and protection of any chemical
functionalities, synthesis of compounds of struc-

15   tural formula (I) and (II) not specifically set
forth herein can be accomplished by methods analo-
gous to the schemes set forth below.

Unless otherwise noted, all starting mate-
rials were obtained from commercial suppliers and

20   used without further purification. All reactions
and chromatography fractions were analyzed by thin-
layer chromatography on 250-mm silica gel plates,
visualized with UV (ultraviolet) light and I_2 (io-
dine) stain. Flash column chromatography was car-

25   ried out using Biotage 40M silica gel (230-400
mesh). Products and intermediates were purified by
flash chromatography or reverse-phase HPLC.

As illustrated below, the compounds of
general structural formula (I) are prepared by re-

30   acting a compound having the formula,

- 30 -

$$CH_3CCH_2COR^2$$
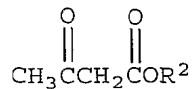
with sodium metal to generate a carbanion, then reacting the carbanion with a compound having the formula

$$(R^3)_n \text{---} \overset{O}{\overset{\|}{C}}\text{-CH}_2\text{Hal}$$
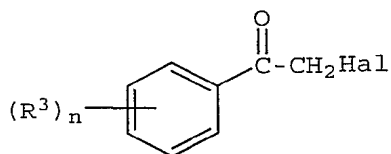
wherein Hal is a halo, typically bromo, to generate an intermediate compound having the formula
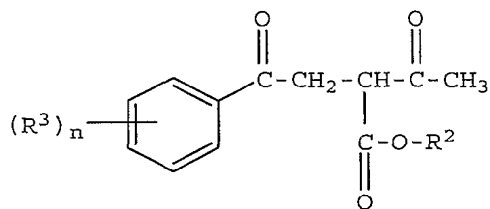
$$(R^3)_n \text{---} \overset{O}{\overset{\|}{C}}\text{-CH}_2\text{-CH-}\overset{O}{\overset{\|}{C}}\text{-CH}_3$$
$$\underset{\overset{\|}{O}}{\overset{\|}{C}}\text{-O-R}^2$$

This intermediate compound then is reacted with a primary amine having the formula R^1NH_2 to provide a compound of structural formula (I).

A compound of structural formula (II) is prepared by reacting a compound having the formula

- 31 -

$$\underset{(R^3)_n}{\longmapsto} \overset{\displaystyle O \quad\quad O}{\underset{\displaystyle \;}{\text{C-CH}_2\text{-C-OR}^2}}$$
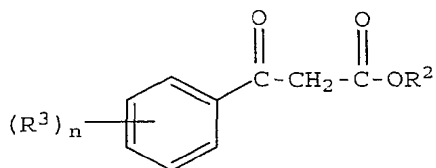
with chloroacetone to generate an intermediate com-
pound having the formula
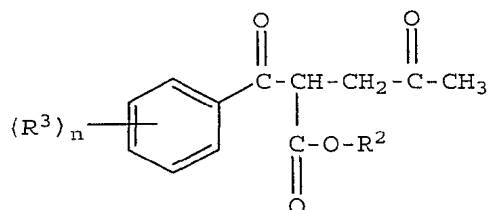
$$\underset{(R^3)_n}{\longmapsto} \text{C-CH-CH}_2\text{-C-CH}_3$$

This intermediate compound then is reacted with a
primary amine having the formula R^1NH_2 to provide a
compound of structural formula (II).

<u>Intermediate 1</u>

**Preparation of 3-Oxo-2-(2-oxo-2-phenyl-ethyl)-
butyric acid ethyl ester**
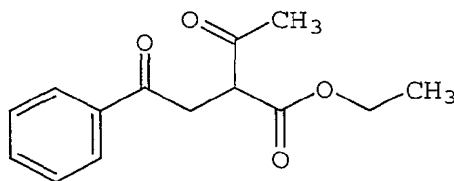
Sodium metal (60mmol, 1.4g) was suspended in 80mL
(milliliter) toluene under nitrogen. Ethyl aceto-
acetate (88mmol (millimole), 11.4g) was added drop-

wise, and the resulting mixture was stirred until all sodium was consumed. The reaction mixture then was cooled in an ice bath (0-5°C) and 2-bromoaceto-phenone (60mmol, 12g) in 140mL toluene was added

5   dropwise. The reaction was stirred overnight with gradual warming to room temperature. Analysis of the reaction mixture by GC and TLC showed that the reaction was complete. The reaction mixture then was washed with 200mL water, then the organic and

10 aqueous layers were separated. The organic layer was dried over $MgSO_4$ (magnesium sulfate). The tolu-ene was removed from the organic layer under vacuum to provide a yellow oil which was purified by Kugelrohr distillation (b.p. 135-145°C at 0.35

15 Torr.).

Intermediate 2

Preparation of 2-Benzoyl-4-oxo-pentanoic acid ethyl ester

20

25

30
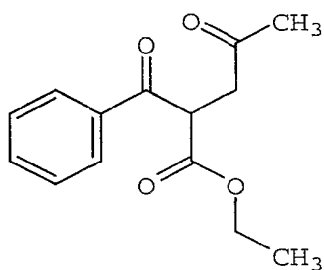
Potassium carbonate (200mmol, 27.6g) was added to a solution of ethyl benzoylacetate (100mmol, 19.2g) and potassium iodide (20mmol, 3.3g) in dry acetone

35 (100mL). The resulting solution was refluxed, and chloroacetone (110mmol, 10.7g) was added dropwise. Analysis of the reaction mixture by GC/MS indicated

the presence of Intermediate 2. Solid material was
filtered from the reaction mixture, then washed with
200mL acetone. The resulting filtrate was concen-
trated under vacuum, redissolved in ether, then
washed three times with water (100mL). The organic
layer was separated from the aqueous layer, and
dried over MgSO$_4$. The solids were separated, and the
organic solvents removed under vacuum to yield a
yellow/orange oil. The crude oil was purified by
Kugelrohr distillation (b.p. 175-185 at 0.65 Torr.).

Example 1

Preparation of 1-(3-Dimethylaminophenyl)-2-methyl-5-
phenyl-1H-pyrrole-3-carboxylic acid ethyl ester
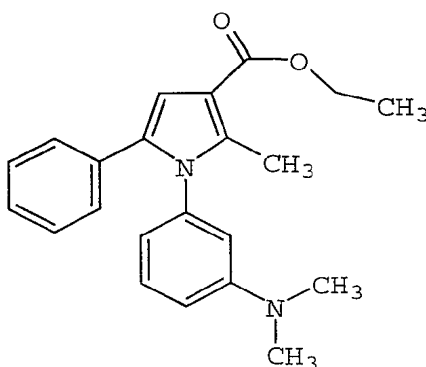
N,N-dimethyl-1,3-phenylenediamine dihydrochloride
(5mmol, 1.0g), triethylamine (10mmol, 1.4mL), 3-oxo-
2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester
(5mmol, 1.2g), and tosic acid (0.1g) were combined
in ethanol (25mL), then heated under reflux (12
hrs). The solvents were removed under vacuum, the
resulting oil was redissolved in methylene chloride,
then applied to a silica column. The purified prod-
uct was collected as a translucent oil. The named

- 34 -

compound was identified by ^{1}H-NMR (CDCl$_3$, ppm): 1.28
t (3H), 2.35 s (3H), 2.77 s (6H), 4.2 q (2H), 6.3-
6.7 m (4H), and 7.0-7.3 m (5H).

5   Example 2

Preparation of 1-(3-Chlorophenyl)-5-methyl-2-phenyl-
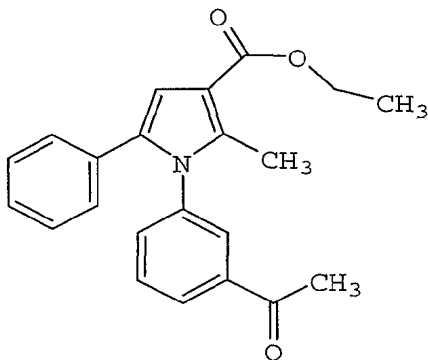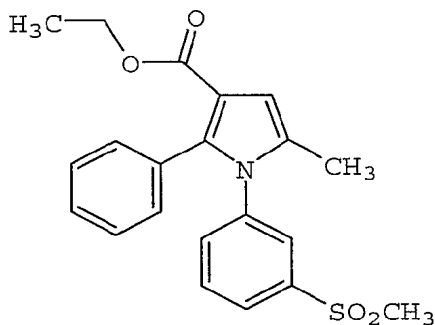1H-pyrrole-3-carboxylic acid ethyl ester
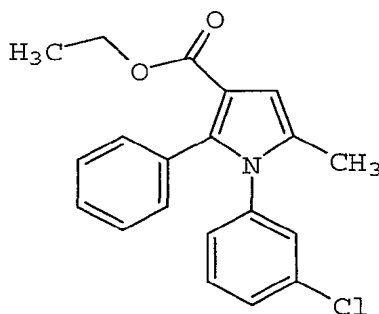
m-Chloroaniline (5mmol, 0.6g), 2-benzoyl-4-oxo-
pentanoic acid ethyl ester (5mmol, 1.2g), and tosic
acid (0.1g) were combined in ethanol, then heated
25   under reflux. Upon completion of the reaction, the
crude solid was recrystallized from ethanol to pro-
vide the named product (m.p. 93.2°C-94.6°C).

- 35 -

Example 3

Preparation of 1-(3-Chlorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15

20
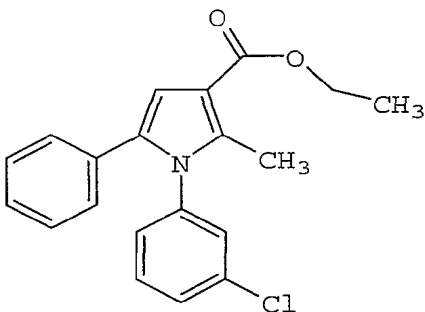
m-Chloroaniline (5mmol, 0.6g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. Upon completion of
25 the reaction, the crude solid was recrystallized from ethanol to afford the desired product (m.p. 99.6°C-100.5°C).

Example 4

Preparation of 5-Methyl-2-phenyl-1-m-tolyl-1H-
pyrrole-3-carboxylic acid ethyl ester
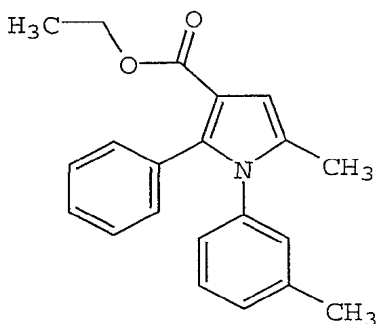
m-Toluidine (5mmol, 0.5g), 2-benzoyl-4-oxo-pentanoic
acid ethyl ester (5mmol, 1.2g), and tosic acid
(0.1g) were combined in ethanol, then heated under
reflux. The resulting oil was resuspended in methy-
lene chloride, then washed with water, 5% sodium
bicarbonate, and brine. The organic layer was dried
over NaSO$_4$ (sodium sulfate), the solids were sepa-
rated, and the solvents evaporated under vacuum.
The crude product was recrystallized from ethanol to
yield the named product (m.p. 92.5°C-94.0°C).

- 37 -

Example 5

Preparation of 2-Methyl-5-phenyl-1-m-tolyl-1H-pyrrole-3-carboxylic acid ethyl ester

5

10
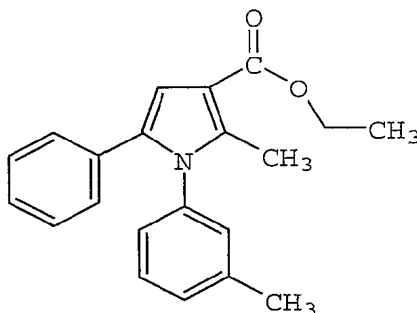
15

m-Toluidine (5mmol, 0.5g), 3-oxo-2-(2-oxo-2-phenyl-
20 ethyl)-butyric acid ethyl ester (5mmol, 1.2g), and
tosic acid (0.1g) were combined in ethanol, then
heated under reflux. The resulting oil was resus-
pended in methylene chloride, then washed with wa-
ter, 5% sodium bicarbonate, and brine. The organic
25 layer was dried over NaSO$_4$, then the solids were
separated and the solvents evaporated under vacuum.
The crude product was recrystallized from ethanol to
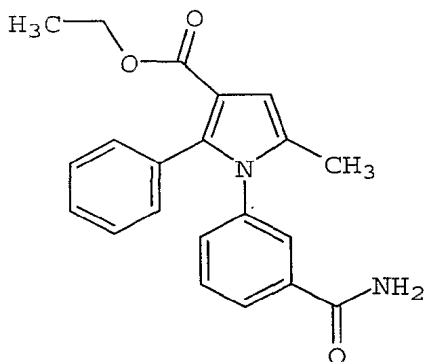yield the named product (m.p. 103°C-104.5°C).

- 38 -

Example 6

Preparation of 1-(3-Bromophenyl)-5-methyl-2-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15
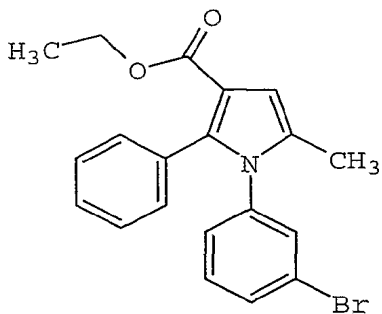
     m-Bromoaniline (5mmol, 0.9g), 2-benzoyl-4-oxo-
pentanoic acid ethyl ester (5mmol, 1.2g), and tosic
20   acid were combined in ethanol, then heated under
reflux. The resulting oil was resuspended in methy-
lene chloride, then washed with water, 5% sodium
bicarbonate and brine. The organic layer was dried
over MgSO$_4$, then the solids were separated and the
25   solvents evaporated under vacuum. The crude product
was recrystallized from ethanol to yield the named
product (m.p. 117.5°C-119.2°C).

- 39 -

Example 7

Preparation of 1-(3-Acetylphenyl)-5-methyl-2-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester

5

10

15
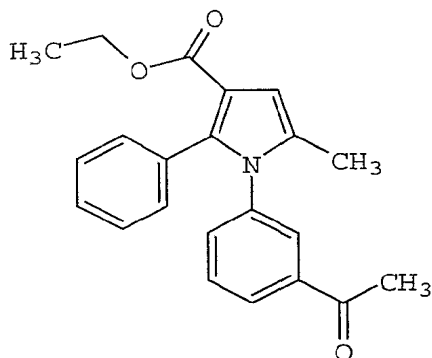
3'-Amino-acetophenone (5mmol, 0.7g), 2-benzoyl-4-
20 oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and
tosic acid (0.1g) were combined in ethanol, then
heated under reflux. The residual oil was purified
over silica and yielded the named product (m.p.
100°C-102°C).

25

Example 8

Preparation of 1-(3-Acetylphenyl)-2-methyl-5-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester

30

35

- 40 -

3'-Aminoacetophenone (5mmol, 0.7g), 3-oxo-2-(2-oxo-
2-phenyl-ethyl)-butyric acid ethyl ester (5mmol,
1.2g), and tosic acid (0.1g) were combined in etha-
nol, then heated under reflux. The resulting oil
5   was purified over silica and yielded the named prod-
uct as a solid (m.p. 102°C-104°C).

Example 9
Preparation of 1-(3-Methanesulfonylphenyl)-5-methyl-
10   **2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester**

15

20

25

3-Methylsulfonylaniline hydrochloride (5.8mmol,
1.2g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester
(5.8mmol, 1.44g), triethylamine (5.8mmol, 0.61g),
30   and tosic acid (0.1g) were combined in ethanol, then
heated under reflux. The resulting oil was resus-
pended in chloroform, then washed with water, 5%
sodium bicarbonate, and brine. The organic layer
was dried over $NaSO_4$, then the solids were separated
35   and solvents removed under vacuum. The crude mate-
rial was purified over silica to yield the named
product (m.p. 131°C-133°C).

- 41 -

Example 10

Preparation of 1-(3-Methanesulfonylphenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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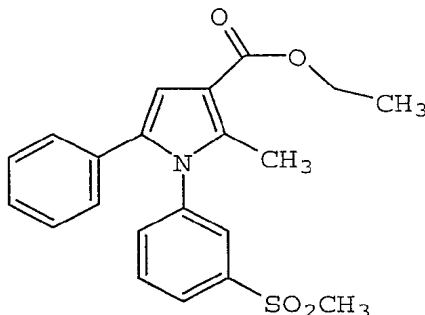
3-Methylsulfonylaniline (1.4mmol, 0.3g), 3-oxo-2-(2-
20 oxo-2-phenyl-ethyl)-butyric acid ethyl ester
    (1.4mmol, 0.35g), triethylamine (1.4mmol, 0.14g),
    and tosic acid (0.1g) were combined in ethanol, then
    heated under reflux. The resulting oil was resus-
    pended in chloroform, then washed with water, 5%
25 sodium bicarbonate, and brine. The organic layer
    was dried over $NaSO_4$, then the solids were separated
    and the solvents removed under vacuum. The crude
    material was purified over silica gel to yield the
    named product as an oil. The structure of the com-
30 pound was verified by ^1H-NMR ($CDCl_3$, ppm): 1.36 t
    (3H), 2.45 s (3H), 2.82 s (3H), 4.3 q (2H), and 6.8-
    7.2 m (9H).

- 42 -

Example 11

Preparation of 1-(3-Carbamoylphenyl)-2-methyl-5-
phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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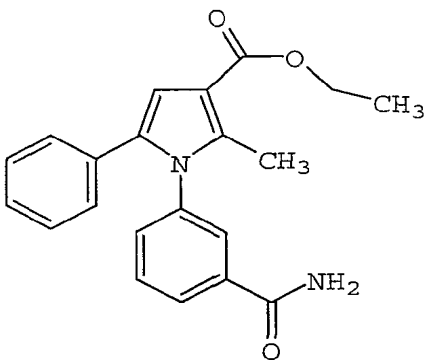
3-Aminobenzamide (5mmol, 1.0g), 3-oxo-2-(2-oxo-2-
20 phenyl-ethyl)-butyric acid ethyl ester (5mmol,
1.2g), and tosic acid (0.1g) were combined in etha-
nol, then heated under reflux. The resulting pre-
cipitate was filtered from the mixture, then washed
with ethanol. The white solid product was used
25 without further purification (m.p. 237.8°C-238.8°C).

Example 12

Preparation of 1-(3-Carbamoylphenyl)-5-methyl-2-
phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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3-Aminobenzamide (5mmol, 1.0g), 2-benzoyl-4-oxo-
pentanoic acid ethyl ester (5mmol, 1.2g), and tosic
acid (0.1g) were combined in ethanol, then heated
under reflux. The resulting precipitate was fil-
25   tered from the reaction mixture, then washed with
ethanol. The tan solid product was used without
further purification (m.p. 208.9°C-210.3°C).

- 44 -

Example 13

Preparation of 1-(3-Dimethylaminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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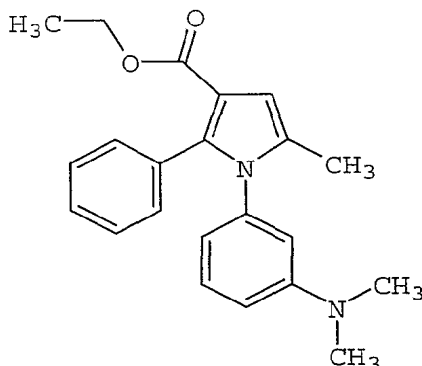
20   N,N-Dimethyl-1,3-phenylenediamine dihydrochloride
     (5mmol, 1.0g), 2-benzoyl-4-oxo-pentanoic acid ethyl
     ester (5mmol, 1.2g), triethylamine (10mmol, 1.0g),
     and tosic acid (0.1g) were combined in ethanol, then
     heated under reflux. The resulting oil was resus-
25   pended in methylene chloride, then washed with wa-
     ter, 5% sodium bicarbonate, and brine. The organic
     layer was dried over $NaSO_4$, then the solids were
     separated and the solvents removed under vacuum.
     The crude material was purified over silica gel and
30   yielded the named product (m.p. 111.7°C-114°C).

- 45 -

Example 14

Preparation of 1-(3-Iodophenyl)-5-methyl-2-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester

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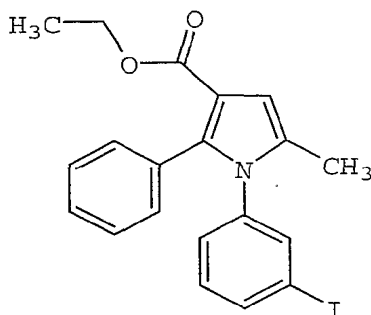
m-Iodoaniline (5mmol, 1.1g), 2-benzoyl-4-oxo-
pentanoic acid ethyl ester (5mmol, 1.2g), and tosic
20   acid (0.1g) were combined in ethanol, then heated
under reflux. The residual oil was resuspended in
methylene chloride, then washed with water, 5% so-
dium bicarbonate, and brine. The solids were sepa-
rated, and the solvents removed under vacuum. The
25   resulting solid was recrystallized in ethanol. A
portion of the material did not dissolve, and was
separated by filtration. Both the recrystallized
and filtered material yielded the named product
(m.p. 142.6°C-144°C).

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- 46 -

Example 15

Preparation of 2-Methyl-5-phenyl-1-(3-sulfophenyl)-
1H-pyrrole-3-carboxylic acid ethyl ester

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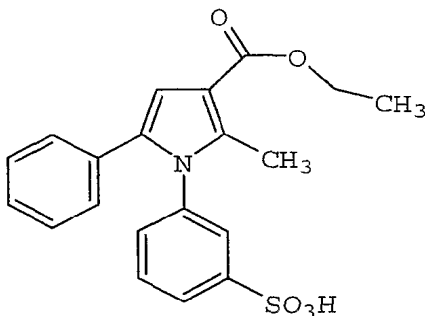
20   Metanilic acid (5mmol, 0.9g), 3-oxo-2-(2-oxo-2-
      phenyl-ethyl)-butyric acid ethyl ester (5mmol,
      1.2g), sodium hydroxide (5mmol, 0.2g), and tosic
      acid (0.1g) were combined in ethanol, then heated
      under reflux. The unreacted metanilic acid was
25   precipitated on ice and filtered from the reaction
      mixture. The filtrate was evaporated to yield an
      oil, which was purified over silica gel. After
      standing, the named product solidified as purple
      crystals (m.p. 94°C-96°C).

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- 47 -

Example 16

Preparation of 5-Methyl-2-phenyl-1-(3-sulfophenyl)-
1H-pyrrole-3-carboxylic acid ethyl ester

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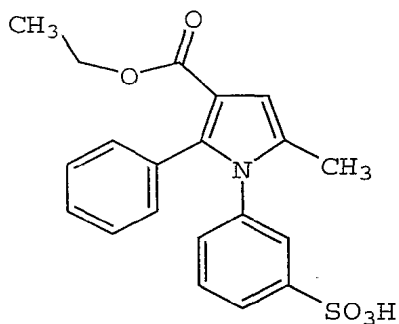
Metanilic acid (5mmol, 0.2g), 2-benzoyl-4-oxo-
pentanoic acid ethyl ester (5mmol, 1.2g), sodium
hydroxide (5mmol, 0.2g), and tosic acid (0.1g) were
combined in ethanol, then heated under reflux. The
resulting oil was treated with HCl (hydrochloric
acid) at 0°C to 5°C to precipitate the unreacted
metanilic acid. The remaining material was concen-
trated under vacuum and purified over silica gel.
The named product was collected as brown crystals
(m.p. 91°C-92.5°C).

- 48 -

Example 17

Preparation of 1-(3-Fluorophenyl)-5-methyl-2-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester

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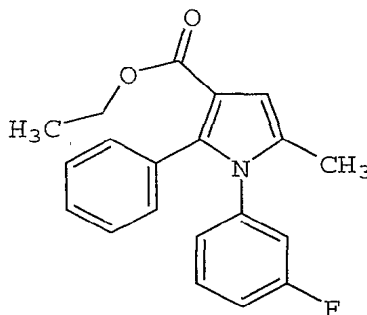
m-Fluoroaniline (5mmol, 0.6g), 2-benzoyl-4-oxo-

20   pentanoic acid ethyl ester (5mmol, 1.2g), and tosic

acid (0.1g) were combined in ethanol, then heated

under reflux. The resulting oil was resuspended in

chloroform, then washed with water, 5% sodium bicar-

bonate, and brine. The organic layer was dried over

25   NaSO$_4$, then the solids filtered away and the solvents

removed under vacuum. The named product was recrys-

tallized from ethanol (m.p. 100°C-101°C).

- 49 -

Example 18

Preparation of 1-(3-Fluorophenyl)-2-methyl-5-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester
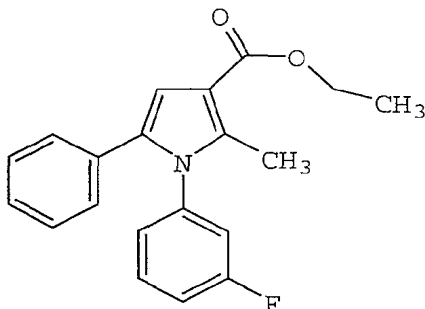
5

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m-Fluoroaniline (5mmol, 0.6g), 3-oxo-2-(2-oxo-2-
phenyl-ethyl)-butyric acid ethyl ester (5mmol,
1.2g), and tosic acid were combined in ethanol, then
20   heated under reflux. The resulting solid was resus-
pended in chloroform, then washed with water, 5%
sodium bicarbonate, and brine. The organic layer
was dried over $NaSO_4$, then the solids were separated
and the solvents removed under vacuum. The named
25   product was recrystallized from ethanol (m.p.
96.3°C-98.0°C).

Example 19

Preparation of 2-Methyl-5-phenyl-1-pyridin-3-yl-1H-
30   pyrrole-3-carboxylic acid ethyl ester

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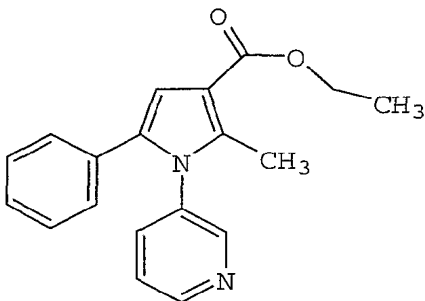
- 50 -

3-Aminopyridine (5mmol, 0.5g), 3-oxo-2-(2-oxo-2-
phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g)
and tosic acid (0.1g) were combined in ethanol, then
5   heated under reflux. The resulting oil was resus-
pended in ether, then washed with water and brine.
The organic layer was dried over $MgSO_4$, then the
solids were separated and the solvents were removed
under vacuum. The named product was purified over
10   silica gel and analyzed by ^{1}H-NMR ($CDCl_3$, ppm): 1.35
t (3H), 2.40 s (3H), 4.3 q (2H), 6.78 s (1H), 7.03-
7.39 m (7H), 8.4 sd (1H), and about 8.6 dd (1H).

- 51 -

Example 20

Preparation of 1-Cyclohexyl-2-methyl-5-phenyl-1H-
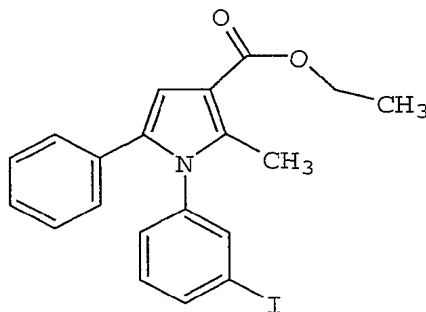pyrrole-3-carboxylic acid ethyl ester
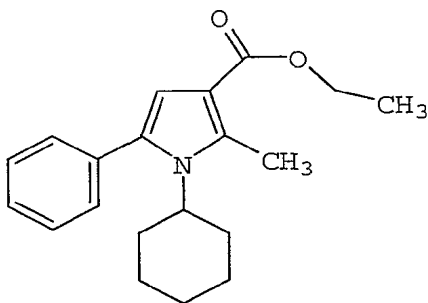
Cyclohexylamine (5mmol, 0.5g), 3-oxo-2-(2-oxo-2-
phenyl-ethyl)-butyric acid ethyl ester (5mmol, 1.2g)
and tosic acid (0.1g) were combined in ethanol, then
heated under reflux. The resulting residue was
resuspended in ether, then washed with water and
brine. The organic layer was dried over $MgSO_4$, and
the solids were separated and the solvents removed
under vacuum. Purification of the oil yielded the
named product, ^{1}H-NMR ($CDCl_3$, ppm): 1.0 bm (2H),
1.23 t (3H), 1.6-1.7 bm (8H), 2.66 s (3H), 4.16 q
(2H), 6.39 s (1H), and 7.25 bm (5H).

- 52 -

Example 21

Preparation of 5-Methyl-1,2-diphenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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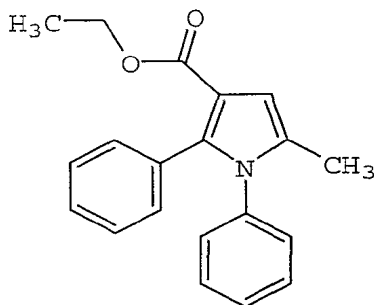
Aniline (10mmol, 0.9g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (10mmol, 2.5g), and tosic acid
20   (0.1g) were combined in ethanol, then heated under reflux. The named product was recrystallized from ethanol (m.p. 130°C-131.7°C).

Example 22

25   Preparation of 2-Methyl-1-(3-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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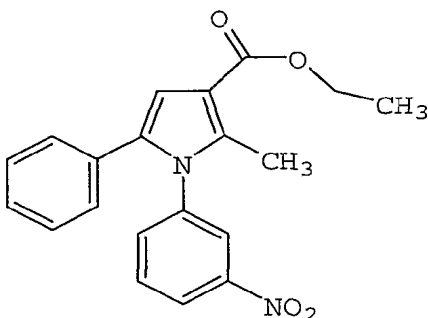
40

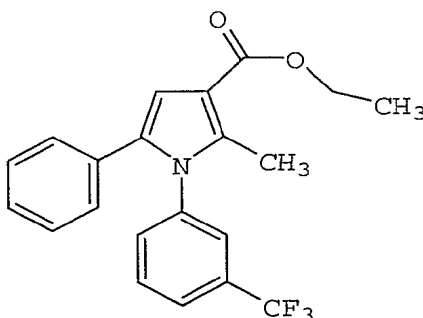m-Nitroaniline (10mmol, 1.4g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (10mmol, 2.5g), and tosic acid (0.1g) were combined in etha-

- 53 -

nol, then heated under reflux. The named product
was recrystallized from ethanol (m.p. 143°C-145°C).

Example 23

5   Preparation of 5-Methyl-1-(5-nitropyridin-2-yl)-2-
phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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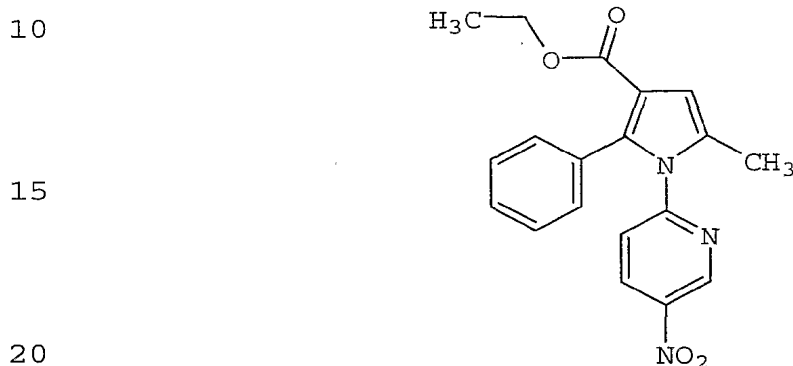
5-Nitro-2-aminopyridine (5mmol, 0.7g), 2-benzoyl-4-
25   oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and
tosic acid (0.1g) were combined in ethanol, then
heated under reflux. The resulting solid was
triturated with chloroform, and the insoluble mate-
rial filtered from the reaction mixture. The fil-
30   trate was purified over silica gel and yielded the
named product (m.p. 146.6°C-148.6°C).

- 54 -

Example 24

Preparation of 1-(3-Carboxyphenyl)-5-methyl-2-
phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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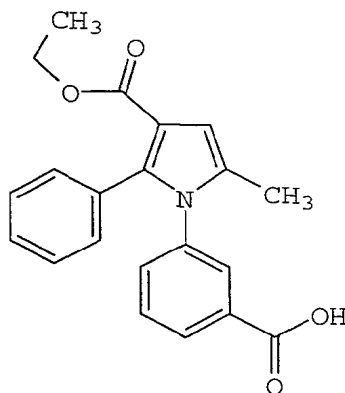
20   3-Aminobenzoic acid (10mmol, 1.4g), 2-benzoyl-4-oxo-
      pentanoic acid ethyl ester (10mmol, 2.5g), and tosic
      acid (0.1g) were combined in ethanol, then heated
      under reflux. The resulting oil was resuspended in
      chloroform-ether, then the insoluble material was
25   filtered from the reaction product. The soluble
      material was purified over silica gel which yielded
      the named product (m.p. 161.0°C-162.7°C).

Example 25

Preparation of 1-(3-Methoxycarbonylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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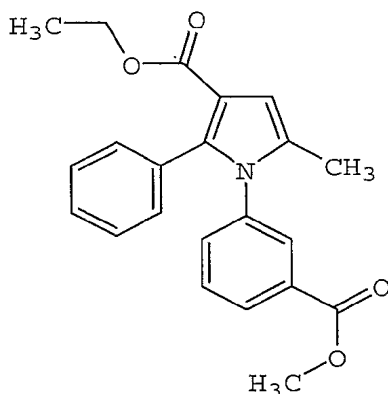
20

Methyl 3-aminobenzoate (10mmol, 1.4g), 2-benzoyl-4-oxo-pentanoic acid ethyl ester (10mmol, 2.5g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The named product was purified
25 over silica gel and crystallized upon standing,
^{1}H-NMR (CDCl$_3$, ppm): 1.15 t (3H), 2.08 s (3H), 3.88 s (3H), 4.05 q (2H), 6.56 s (1H), 7.15-7.33 bm (7H), 7.79-7.97 bm (2H).

- 56 -

Example 26

Preparation of 1-(3-Bromophenyl)-2-methyl-5-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester

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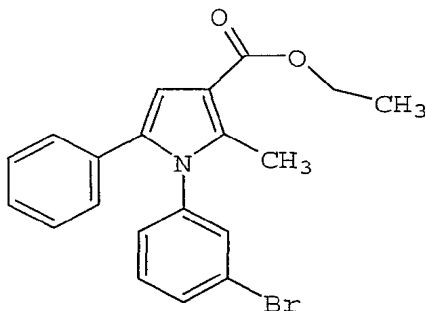
m-Bromoaniline (5mmol, 0.9g), 3-oxo-2-(2-oxo-2-
phenyl-ethyl)-butyric acid ethyl ester (5mmol,

20   1.2g), and tosic acid (0.1g) were combined in etha-
nol, then heated under reflux. The named product
was recrystallized from ethanol (m.p. 111°C-
112.7°C).

25   Example 27

Preparation of 1-(3-Iodophenyl)-2-methyl-5-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester

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m-Iodoaniline (5mmol, 1.1g), 3-Oxo-2-(2-oxo-2-
phenyl-ethyl)-butyric acid ethyl ester (5mmol,

- 57 -

1.2g), and tosic acid (0.1g) were combined in etha-
nol, then heated under reflux. The named product
was recrystallized in ethanol (m.p. 111°C-112.9°C).

5   <u>Example 28</u>

<u>Preparation of 2-Methyl-5-phenyl-1-(3-trifluoro-
methylphenyl)-1H-pyrrole-3-carboxylic acid ethyl
ester</u>

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m-Trifluoromethylaniline (5mmol, 0.8g), 3-oxo-2-(2-
25   oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol,
1.2g), and tosic acid (0.1g) were combined in etha-
nol, then heated under reflux. The crude material
was resuspended in chloroform and washed with water,
then with brine. The organic layer was dried over
30   NaSO$_4$. The solids were separated and the solvents
removed under vacuum. The resulting oil was puri-
fied over silica gel to yield the named product,
^{1}H-NMR (CDCl$_3$, ppm): 1.37 t (3H), 2.42 s (3H), 4.32
q (2H), 6.80 s (1H), 7.0-7.6 bm (9H).

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Example 29

Preparation of 5-Methyl-2-phenyl-1-(3-trifluoro-
methylphenyl)-1H-pyrrole-3-carboxylic acid ethyl
ester
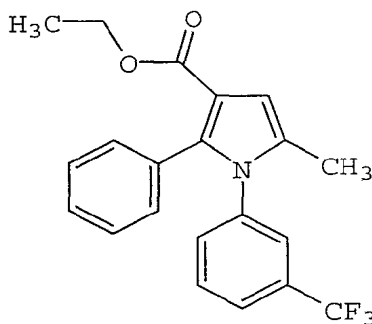
m-Trifluoromethylaniline (5mmol, 0.8g), 2-benzoyl-4-
oxo-pentanoic acid ethyl ester (5mmol, 1.2g), and
tosic acid (0.1g) were combined in ethanol, then
heated under reflux. The crude material was resus-
pended in chloroform, washed with water, then washed
with brine. The organic layer was dried over $NaSO_4$.
The solids were separated and the solvents removed
under vacuum. The resulting oil was purified over
silica gel to yield the named product, ^{1}H-NMR ($CDCl_3$,
ppm): 1.01 t (3H), 1.98 b (3H), 4.00 q (2H), 6.48 s
(1H), 7.00-7.33 bm (9H).

Example 30

Preparation of 2-Methyl-1-(3-nitrobenzyl)-5-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester
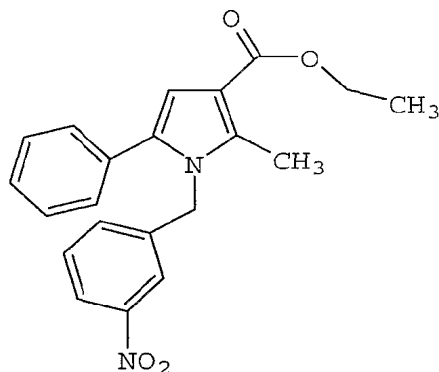
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20   m-Nitrobenzylamine hydrochloride (3g) was suspended
      in an aqueous solution of saturated sodium bicarbon-
      ate (30mL), then extracted with chloroform. The
      organic layer was dried over MgSO$_4$, the solids sepa-
      rated and solvents removed under vacuum to yield the
25   free base. The m-nitrobenzylamine (5mmol, 0.8g)
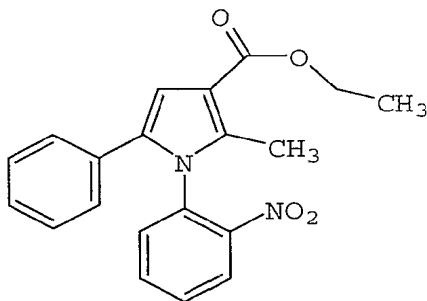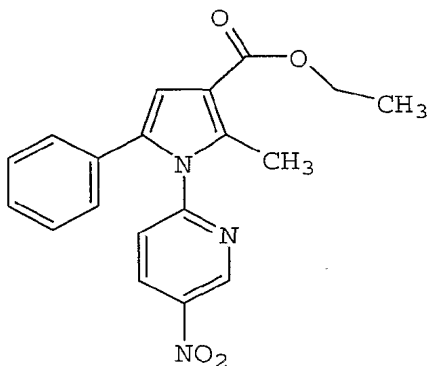   prepared above, 3-oxo-2-(2-oxo-2-phenyl-ethyl)-bu-
      tyric acid ethyl ester (5mmol, 1.2g), and tosic acid
      (0.1g) were combined in ethanol, then heated under
      reflux. The residue was resuspended in ethyl ether,
30   and the benzylamine tosic acid salt was filtered
      from the mixture. The filtrate was concentrated
      under vacuum, and the resulting oil purified over
      silica to yield the named product, ^{1}H-NMR (CDCl$_3$,
      ppm): 1.24 t (3H), 2.36 s (3H), 4.07-4.30 q (2H),
35   5.11 s (2H), 6.58 s (1H), 6.98-7.44 bm (7H), 7.69 bs
      (1H), 7.97 bd (1H).

- 60 -

Example 31

Preparation of 5-Methyl-1-(3-nitrobenzyl)-2-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester
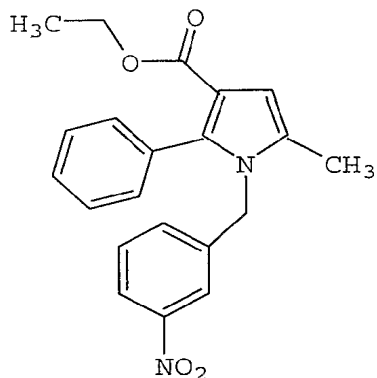
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m-Nitrobenzylamine hydrochloride (3g) was suspended
in an aqueous solution of saturated sodium bicarbon-
ate (30mL), and extracted with chloroform. The
organic layer was dried over MgSO$_4$, the solids satu-
25   rated and solvents removed under vacuum to yield the
free base. The m-nitrobenzylamine (5mmol, 0,8g)
prepared above, 2-benzoyl-4-oxo-pentanoic acid ethyl
ester (5mmol, 1.2g), and tosic acid were combined in
ethanol, then heated under reflux. The resulting
30   residue was purified over silica gel to yield the
named product, ^{1}H-NMR (CDCl$_3$, ppm): 1.10 t (3H),
3.16 s (3H), 4.08 q (2H), 5.04 s (2H), 6.55 s (1H),
7.07-7.53 bm (7H), 7.72 bs (1H), 8.04 bd (1H).

- 61 -

Example 32

Preparation of 5-Methyl-2-phenyl-1-pyridin-3-yl-1H-pyrrole-3-carboxylic acid ethyl ester

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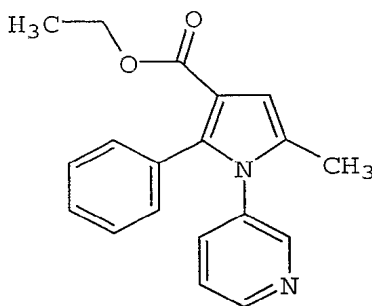
3-Aminopyridine (5mmol, 0.5g), 2-benzoyl-4-oxo-
20   pentanoic acid ethyl ester (5mmol, 1.2g), and tosic
      acid (0.1g) were combined in ethanol, then heated
      under reflux. The resulting oil was purified over
      silica, but still contained contaminants. This
      material was triturated in hexane, and the solids
25   were separated by filtration. The solids were
      washed with benzene two times to yield the named
      product (m.p. 102°C-103°C).

Example 33

Preparation of 2-Methyl-1-(4-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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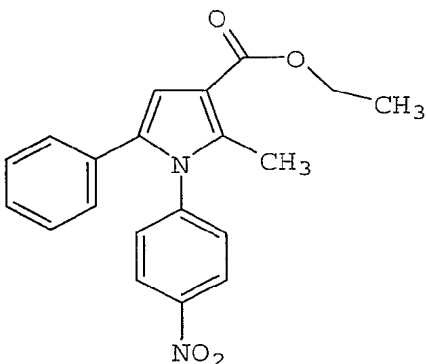
p-Nitroaniline (5mmol, 0.7g), 3-oxo-2-(2-oxo-2-
20   phenyl-ethyl)-butyric acid ethyl ester (5mmol,
1.25g), and tosic acid were combined in ethanol,
then heated under reflux. The reaction mixture was
cooled on ice (0-5°C), and the precipitate filtered
from the reaction mixture. ^{1}H-NMR analysis confirmed
25   that the precipitate was the named product, (CDCl$_3$,
ppm); 1.38 t (3H), 2.45 s (3H), 4.20 q (2H), 6.80 s
(1H), 6.97-7.35 bm (7H), 8.25 d (2H).

- 63 -

Example 34

Preparation of 2-Methyl-1-(2-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

o-Nitroaniline (15mmol, 2.1g), 3-oxo-2-(2-oxo-2-phenyl-ethyl)-butyric acid ethyl ester (15mmol, 3.7g), and tosic acid (0.1g) were combined in ethanol, then heated under reflux. The resulting oil was purified over three silica gel columns to yield the named product, ^{1}H-NMR (CDCl$_3$, ppm): 1.36 t (3H), 2.36 s (3H), 4.31 q (2H), 6.80 s (1H), 7.03-7.66 bm (6H), 7.68 dt (2H), 7.97 dd (1H).

Example 35

Preparation of 2-Methyl-1-(5-nitropyridin-2-yl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

- 64 -

5-Nitro-pyridin-2-ylamine (5mmol, 0.7g), 3-oxo-2-(2-
oxo-2-phenyl-ethyl)-butyric acid ethyl ester (5mmol,
1.2g), and tosic acid (0.1g) were combined in etha-
nol, then heated under reflux. The resulting solid
5   was triturated with chloroform, the filtrate was
further purified over silica gel to yield the named
product, ^{1}H-NMR (CDCl$_3$, ppm): 1.35 t (3H), 2.54 s
(3H), 4.30 q (2H), 6.78 s (1H), 6.96-7.25 bm (6H),
8.33 dd (1H), 9.39 sd (1H).

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Example 36

**Preparation of 5-Methyl-1-(4-nitrophenyl)-2-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester**

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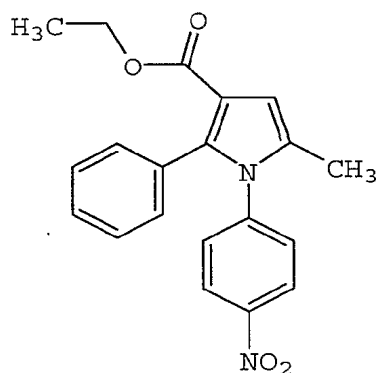
30   p-Nitroaniline (10mmol, 1.4g), 2-benzoyl-4-oxo-
pentanoic acid ethyl ester (10mmol, 2.5g), and tosic
acid (0.1g) were combined in ethanol, then heated
under reflux. The reaction mixture was filtered and
the solids washed with ethanol. The collected mate-
35   rial was determined to be the named product (m.p.
190°C-192°C).

- 65 -

Example 37

Preparation of 1-(4-Aminophenyl)-5-methyl-2-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester

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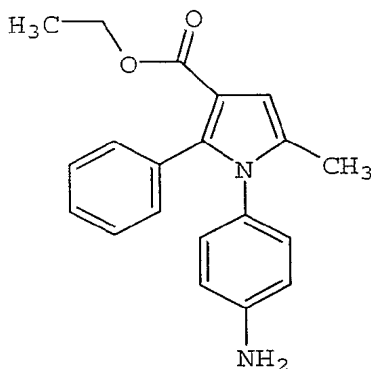
20 5-Methyl-1-(4-nitrophenyl)-2-phenyl-1H-pyrrole-3-
    carboxylic acid ethyl ester of Example 36 (4mmol,
    1.5g) was dissolved in ethanol, and treated with 5%
    Pd/C (0.15g). The mixture was hydrogenated with
    H_2(g) at 45 psi. Upon completion of the reaction,
25 the mixture was filtered through Celite, and the
    solvents removed under vacuum. Analysis of the
    resulting solid showed it was the named product
    (m.p. 181°C-183°C).

- 66 -

Example 38

Preparation of 1-(4-Aminophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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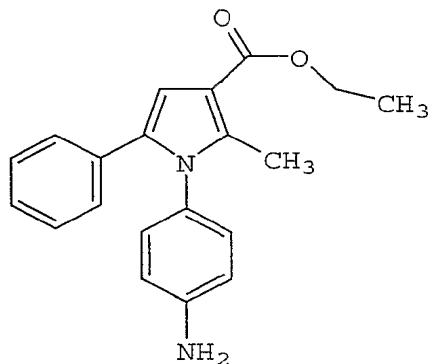
2-Methyl-1-(4-nitro-phenyl)-5-phenyl-1H-pyrrole-3-
20   carboxylic acid ethyl ester of Example 33 (2mmol,
      0.8g) was dissolved 50% solution of ethyl acetate in
      ethanol, and treated with Pd/C (0.088g). The mix-
      ture was hydrogenated with H_2(g) at 45 psi. Upon
      completion of the reaction, the mixture was filtered
25   through Celite and the solvents removed under vac-
      uum. This material was purified over silica gel to
      yield the named product.

- 67 -

Example 39

1-(3-Nitrophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester
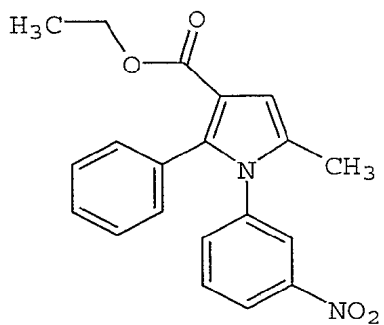
The named product was purchased from Maybridge Chemical Co., Ltd., Cornwall, UK, and used without further purification.
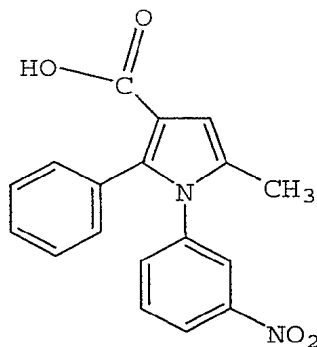
Example 40

Preparation of 1-(3-Aminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester
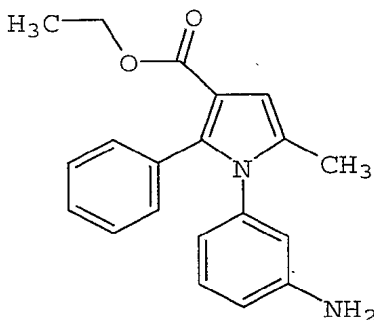

The compound of Example 39 (0.86mmol, 0.3g) was dissolved in methanol, and treated with nickel chloride ($NiCl_2$) and sodium borohydride ($NaBH_4$) (17.2mmol). The methanol was removed, and then the reaction mixture was resuspended in water, and next extracted with ethyl acetate. The reaction mixture was dried over $MgSO_4$, filtered, and concentrated. The named product was purified over silica. ^{1}H-NMR ($CDCl_3$, ppm): 1.37 t (3H), 2.41 s (3H), 3.73 s (2H), 4.31 q (2H), 6.43 s (1H), 6.54 d (1H), 6.67 dd (1H), 6.78 s (1H), 7.11-7.18 m (5H).

Example 41

Preparation of 1-(3-Aminophenyl)-2-methyl-5-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester

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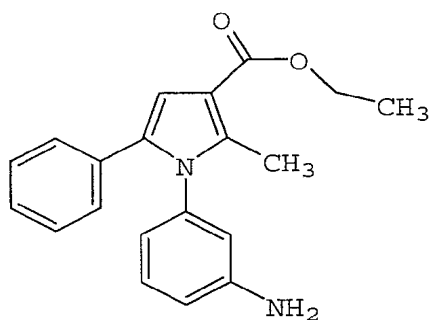
20   The compound of Example 22 (0.86mmol,
0.3g) was dissolved in methanol and treated with
NiCl$_2$ and NaBH$_4$ (17.2mmol) using the procedure set
forth in Example 40. The named product was purified
over silica (m.p. 142°C-143°C).

25

Example 42

1-(2,3-Dichlorophenyl)-5-methyl-2-phenyl-
1H-pyrrole-3-carboxylic acid ethyl ester

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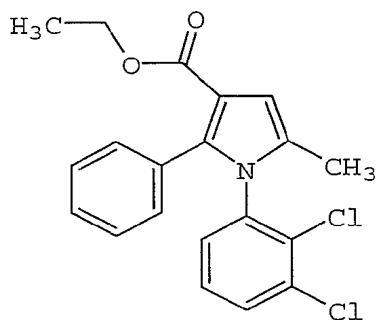
- 70 -

The named product was purchased from
Maybridge Chemical Co., Ltd., and used without fur-
ther purification.

5   **Example 43**

Preparation of 5-Methyl-1-(3-nitrophenyl)-
2-phenyl-1H-pyrrole-3-carboxylic acid

The compound of Example 39 (0.29mmol,

25   0.1g) was dissolved in a 50% dioxane and water, then
treated with lithium hydroxide (LiOH, 0.87mmol,
0.0363g) and heated to at least 50°C. The solvents
were removed and the base extracted with ethyl ace-
tate. The aqueous layer was acidified to pH<2 and

30   extracted with ethyl acetate. The organic layer was
dried over MgSO$_4$, the solids filtered, and the sol-
vents removed under vacuum. The named product was
used without further purification. ^{1}H-NMR (CDCl$_3$,
ppm): 2.48 s (3H), 6.88 s (1H), 7.03 dd (2H), 7.18

35   m (3H), 7.47 dd (1H), 7.6 t (1H), 8.08 st (1H), 8.27
dd (1H).

- 71 -

Example 44

Preparation of 5-Methyl-1,2-diphenyl-1H-pyrrole-3-carboxylic acid

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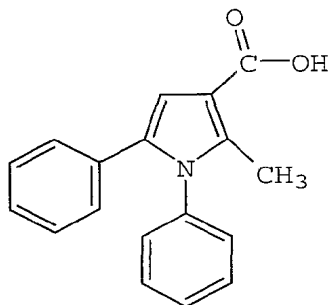
The compound of Example 21 (0.33mmol, 0.1g) was hydrolyzed in the same manner as set forth in Example 43 to yield the named product, ^{1}H-NMR (CDCl$_3$ ppm):   2.44 s (3H), 6.87 s (1H), 7.06 m (2H), 7.15 m (5H), 7.40 m (3H).

20

Example 45

1-(3,5-Bis-trifluoromethylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester
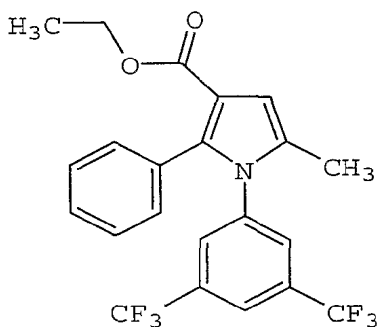
25

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The named product was purchased from Maybridge Chemical Co., Ltd., and used without further purification.

- 72 -

Example 46

Preparation of 1-(3-Ethoxycarbonylphenyl)-5-methyl-
2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester

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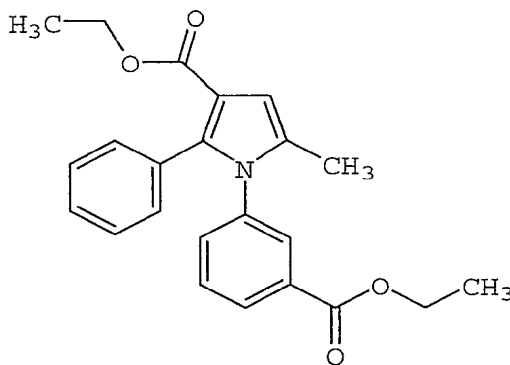
20   This compound was formed in an identical
manner as the compound of Example 25, except ethyl
3-aminobenzoate was used as a starting material.
The named product had an m.p. of 108°C-110°C.

25   The compounds of structural formulae (I)
and (II) were tested for an ability to inhibit PDE4.
The ability of a compound to inhibit PDE4 activity
is related to the IC_{50} value for the compound, i.e.,
the concentration of inhibitor required for 50%
30   inhibition of enzyme activity. The IC_{50} value for
compounds of structural formulae (I) and (II) were
determined using human recombinant PDE4.
        The compounds of the present invention
typically exhibit an IC_{50} value against recombinant
35   human PDE4 of less than about 100 μM, and preferably
less than about 50 μM, and more preferably less than
about 25 μm. The compounds of the present invention
typically exhibit an IC_{50} value against recombinant
human PDE4 of less than about 10 μM, and often less

- 73 -

than about 1 μM. To achieve the full advantage of
the present invention, a present PDE4 inhibitor has
an IC_{50} of about 0.005 μM to about 25 μM.

5   The IC_{50} values for the compounds were
determined from concentration-response curves typi-
cally using concentrations ranging from 0.1 pM to
500 μM. Tests against other PDE enzymes using stan-
dard methodology, as described in Loughney et al.,
J. Biol. Chem., 271, pp. 796-806 (1996), also showed
10   that compounds of the present invention are highly
selective for the cAMP-specific PDE4 enzyme.

In particular, a compound of the present
invention, i.e., Example 22, has an IC_{50} vs. human
recombinant PDE4B of 0.15 μM, but has an IC_{50} vs.
15   PDE1A of 35.5 μM, vs. PDE1B of 21.0 μM, vs. PDE1C of
21.8 μM, vs. PDE2 of 4.5 μM, vs. PDE3A of 89 μM, vs.
PDE5 of 12.5 μM, and vs. PDE7 of 10.6 μM. This
illustrates the selectivity of the present compound
with respect to inhibiting PDE4.

20   The compounds of structural formulae (I)
and (II) also were tested for an ability to reduce
TNFα secretion in human peripheral blood lympho-
cytes. The ability to reduce TNFα secretion is
related to the EC_{50} values (i.e., the effective con-
25   centration of the compound capable of inhibiting 50%
of the total TNFα).

The compounds of the present invention
typically exhibit an EC_{50} value of less than about
100 μM, and preferably less than about 50 μM, and
30   more preferably less than about 15 μM. The com-
pounds of the present invention preferably exhibit a
PBL/TNFα EC_{50} value of less than about 10 μM, and
often less than about 7 μM. To achieve the full

- 74 -

advantage of the present invention, a present PDE4
inhibitor has an EC_{50} value of about 1 μM to about 20
μM.

The production of recombinant human PDEs

5   and the IC_{50} and EC_{50} determinations can be accom-
plished by well-known methods in the art. Exemplary
methods are described as follows:

EXPRESSION OF HUMAN PDEs

10

Expression in Baculovirus-Infected
***Spodoptera fugiperda* (Sf9) Cells**

15   Baculovirus transfer plasmids were con-
structed using either pBlueBacIII (Invitrogen) or
pFastBac (BRL-Gibco). The structure of all plasmids
was verified by sequencing across the vector junc-
tions and by fully sequencing all regions generated

20   by PCR. Plasmid pBB-PDE1A3/6 contained the complete
open reading frame of PDE1A3 (Loughney et al., *J.*
Biol. Chem., *271*, pp. 796-806 (1996)) in pBlue-
BacIII. Plasmid Hcam3aBB contained the complete
open reading frame of PDE1C3 (Loughney et al.

25   (1996)) in pBlueBacIII. Plasmid pBB-PDE3A contained
the complete open reading frame of PDE3A (Meacci et
al., *Proc. Natl. Acad. Sci.*, *USA*, *89*, pp. 3721-3725
(1992)) in pBlueBacIII.

Recombinant virus stocks were produced

30   using either the MaxBac system (Invitrogen) or the
FastBac system (Gibco-BRL) according to the manufac-
turer's protocols. In both cases, expression of
recombinant human PDEs in the resultant viruses was
driven off the viral polyhedron promoter. When

- 75 -

using the MaxBac® system, virus was plaque purified
twice in order to insure that no wild type (occ+)
virus contaminated the preparation. Protein expres-
sion was carried out as follows. Sf9 cells were

5   grown at 27°C in Grace's Insect culture medium
(Gibco-BRL) supplemented with 10% fetal bovine se-
rum, 0.33% TC yeastolate, 0.33% lactalbumin hydro-
lysate, 4.2 mM NaHCO$_3$, 10 μg/mL gentamycin, 100
units/mL penicillin, and 100 μg/mL streptomycin.

10   Exponentially growing cells were infected at a mul-
tiplicity of approximately 2 to 3 virus particles
per cell and incubated for 48 hours. Cells were
collected by centrifugation, washed with nonsup-
plemented Grace's medium, and quick-frozen for stor-

15   age.

Expression in *Saccharomyces cerevisiae* (Yeast)

Recombinant production of human PDE1B,

20   PDE2, PDE4A, PDE4B, PDE4C, PDE4D, PDE5, and PDE7 was
carried out similarly to that described in Example 7
of U.S. Patent No. 5,702,936, incorporated herein by
reference, except that the yeast transformation
vector employed, which is derived from the basic

25   ADH2 plasmid described in Price et al., *Methods in
Enzymology*, 185, pp. 308-318 (1990), incorporated
yeast ADH2 promoter and terminator sequences and the
Saccharomyces cerevisiae host was the protease-defi-
cient strain BJ2-54 deposited on August 31, 1998

30   with the American Type Culture Collection, Manassas,
Virginia, under accession number ATCC 74465. Trans-
formed host cells were grown in 2X SC-leu medium, pH
6.2, with trace metals, and vitamins. After 24

- 76 -

hours, YEP medium-containing glycerol was added to a
final concentration of 2X YET/3% glycerol. Approxi-
mately 24 hr later, cells were harvested, washed,
and stored at -70°C.

CALMODULIN PURIFICATION

Calmodulin used for activation of the PDE1
enzymes was purified from bovine testes essentially
as described by Dedman et al., *Methods in Enzym-
ology, 102*, pp. 1-8 (1983) using the Pharmacia
Phenyl-Sepharose® procedure.

IMMOBILIZATION OF CALMODULIN ON AGAROSE

Calmodulin was immobilized on BioRad Affi-
Gel® 15 per manufacturer's instructions.

HUMAN PHOSPHODIESTERASE PREPARATIONS

Phosphodiesterase Activity Determinations

Phosphodiesterase activity of the prepara-
tions was determined as follows. PDE assays utiliz-
ing a charcoal separation technique were performed
essentially as described in Loughney et al. (1996).
In this assay, PDE activity converts [32P]cAMP or
[32P]cGMP to the corresponding [32P]5'-AMP or
[32P]5'-GMP in proportion to the amount of PDE ac-
tivity present. The [32P]5'-AMP or [32P]5'-GMP then
was quantitatively converted to free [32P]phosphate
and unlabeled adenosine or guanosine by the action
of snake venom 5'-nucleotidase. Hence, the amount

of [32P]phosphate liberated is proportional to en-
zyme activity. The assay was performed at 30°C in a
100 μL reaction mixture containing (final concentra-
tions) 40 mM Tris HCl (pH 8.0), 1 μM ZnSO$_4$, 5 mM

5   MgCl$_2$, and 0.1 mg/mL bovine serum albumin (BSA).
Alternatively, in assays assessing PDE1-specific
activity, incubation mixtures further incorporated
the use of 0.1 mM CaCl$_2$ and 10 μg/mL calmodulin.
PDE enzyme was present in quantities that yield <30%

10   total hydrolysis of substrate (linear assay condi-
tions). The assay was initiated by addition of
substrate (1 mM [32P]cAMP or cGMP), and the mixture
was incubated for 12 minutes. Seventy-five (75) μg
of Crotalus atrox venom then was added, and the

15   incubation was continued for 3 minutes (15 minutes
total). The reaction was stopped by addition of 200
μL of activated charcoal (25 mg/mL suspension in 0.1
M NaH$_2$PO$_4$, pH 4). After centrifugation (750 X g for
3 minutes) to sediment the charcoal, a sample of the

20   supernatant was taken for radioactivity determina-
tion in a scintillation counter and the PDE activity
was calculated.

Inhibitor analyses were performed simi-
larly to the method described in Loughney et al., *J.*

25   *Biol. Chem.*, 271, pp. 796-806 (1996), except both
cGMP and cAMP were used, and substrate concentra-
tions were kept below 32 nM, which is far below the
Km of the tested PDE5.

30   **Purification of PDE1A3 from SF9 Cells**

Cell pellets (5 g) were mixed with 10 mL
of Lysis Buffer (50 mM MOPS pH 7.5, 2 mM dithio-

- 78 -

threitol (DTT), 2 mM benzamidine HCl, 5 μM ZnSO$_4$,
0.1 mM CaCl$_2$, 20 μg/mL calpain inhibitors I and II,
and 5 μg/mL each of leupeptin, pepstatin, and
aprotinin) at room temperature. The cells were
5   lysed by passage through a French® pressure cell
(SLM-Aminco®, Spectronic Instruments, Inc., Roches-
ter NY). The resultant lysate was centrifuged in a
Beckman ultracentrifuge using a type T180 rotor at
45,000 rpm for 1 hr. The supernatant was recovered
10   and filtered through a 0.2 μm filter. This filtrate
was applied to a 2.6 X 90 cm column of SEPHACRYL®
S-300 equilibrated in Column Buffer A (Lysis Buffer
containing 100 mM NaCl, and 2 mM MgCl$_2$). The column
flow rate was adjusted to 1 mL/min and fractions of
15   7 mL were collected. Active fractions were pooled
and supplemented with 0.16 mg of calmodulin. The
enzyme was applied overnight at a flow rate of 0.2
mL/min to an ACC-1 agarose immunoaffinity column as
described in Hansen et al., *Methods in Enzymology*
20   *159*, pp. 453-557 (1988). The column was washed with
5 volumes of Column Buffer B (Column Buffer A with-
out NaCl) and followed by 5 volumes of Column Buffer
C (Column Buffer A containing 250 mM NaCl). The
column was eluted with Column Buffer D (50 mM MOPS
25   pH 7.5, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM
benzamidine HCl, 100 mM NaCl, 20 μg/mL calpain in-
hibitors I and II, and 5 μg/mL each of leupeptin,
pepstatin, and aprotinin) by applying one column
volume at 0.1 mL/min, stopping flow for 1 hour, and
30   then continuing elution at the same flow rate.
Fractions of 0.5 mL were collected. Fractions dis-
playing activity were pooled, and first dialyzed
against dialysis buffer containing 25 mM MOPS pH

- 79 -

7.5, 100 mM NaCl, 10 μM ZnSO$_4$, 1 mM CaCl$_2$, 1 mM DTT, and 1 mM benzamidine HCl. A subsequent dialysis against dialysis buffer containing 50% glycerol was performed prior to quick-freezing the sample with

5   dry ice and storage at -70°C. The resultant preparations were about 10 to 15% pure by SDS-PAGE. These preparations had specific activities of about 5 to 20 μmol cAMP hydrolyzed per minute per milligram protein.

10

Purification of PDE1B from *S. cerevisiae*

Yeast cells (50 g) were thawed by mixing with 100 mL glass beads (0.5 mM, acid washed) and

15   200 mL Buffer A at room temperature. Buffer A consisted of 50 mM MOPS pH 7.5, 1 mM DTT, 2 mM benzamidine HCl, 0.01 mM ZnSO$_4$, 5 mM MgCl$_2$, 20 μg/mL calpain inhibitors I and II, and 5 μg/mL each of leupeptin, pepstatin, and aprotinin. The mixture

20   was cooled to 4°C, transferred to a Bead-Beater®, and the cells lysed by rapid mixing for 6 cycles of 30 seconds each. The homogenate was centrifuged for 15 minutes in a Beckman J2-21M centrifuge using a JA-10 rotor at 9,000 rpm and 4°C. The supernatant

25   was recovered and centrifuged in a Beckman XL-80 ultracentrifuge using a TI45 rotor at 36,000 rpm for 45 minutes at 4°C. The supernatant was recovered and PDE1B was precipitated by the addition of solid ammonium sulfate (0.33 g/mL supernatant) while stir-

30   ring in an ice bath and maintaining the pH between 7.0 and 7.5. This mixture then was centrifuged for 22 minutes in a Beckman J2 centrifuge using a JA-10 rotor at 9,000 rpm (12,000 X g). The supernatant

- 80 -

was discarded and the pellet was dissolved in 100 mL
of buffer B (50 mM MOPS pH 7.5, 1 mM DTT, 1 mM benz-
amidine HCl, 0.01 mM $ZnSO_4$, 2 mM $MgCl_2$, 2 mM $CaCl_2$,
and 5 μg/mL each of leupeptin, pepstatin, and

5   aprotinin). The pH and conductivity were corrected
to 7.5 and 15-20 milliSiemens (mS), respectively.
This solution was loaded onto a 20 mL column of
calmodulin-Agarose that had been equilibrated with
10 column volumes of Buffer B at a rate of 1 mL/min.

10   The flow-through was reapplied to the column at
least 5 times. The column was washed with 5 volumes
of Buffer B, 5 volumes of buffer B containing 250 mM
NaCl, and 2 volumes of Buffer B without NaCl again.
Elution was accomplished by applying one volume of

15   Buffer C (50 mM MOPS pH 7.5, 1 mM EDTA, 1 mM EGTA, 1
mM DTT, 1 mM benzamidine HCl) at 0.33 mL/min, then
stopping flow for 1 hour before continuing the elu-
tion. Fractions of about 4 mL were collected and
assayed for PDE activity. Active fractions were

20   pooled and concentrated to a volume of 5 mL, using
an Amicon ultrafiltration system. The concentrate
was then applied to a 320 mL Sephacryl® S-300 column
(1.6 X 150 cm) that had been equilibrated with at
least 2 volumes of Buffer D (25 mM MOPS pH 7.5, 1 mM

25   DTT, 1 mM benzamidine HCl, 0.01 mM $ZnSO_4$, 2 mM $CaCl_2$,
and 100 mM NaCl). The column was developed at a
flow rate of 1 mL/min (11 cm/hr), and 5 mL fractions
were collected. The activity peak was pooled and
dialyzed overnight against Buffer D containing 50%

30   glycerol. The purified enzyme was frozen on dry ice
and stored at -70°C. The resultant preparations
were about >90% pure by SDS-PAGE. These prepara-

- 81 -

tions had specific activities of about 10 to 30 μmol
cGMP hydrolyzed per minute per milligram protein.

Purification of PDE1C3 from Sf9 Cells

5

            Cell pellets (5 g) were thawed on ice with
20 mL of Lysis Buffer (50 mM MOPS pH 7.4, 10 μM
ZnSO$_4$, 0.1 mM CaCl$_2$, 1 mM DTT, 2 mM benzamidine HCl,
5 μg/mL each of pepstatin, leupeptin, and aprotin-
10   in). Cells were lysed by passage through a French®
pressure cell (SLM-Aminco®, Spectronic Instruments)
while temperatures were maintained below 10°C. The
resultant cell homogenate was centrifuged at 36,000
rpm at 4°C for 45 min in a Beckman ultracentrifuge
15   using a Type TI45 rotor. The supernatant was dis-
carded and the resultant pellet was resuspended with
40 mL of Solubilization Buffer (Lysis Buffer con-
taining 1 M NaCl, 0.1 M MgCl$_2$, 1 mM CaCl$_2$, 20 μg/mL
calmodulin, and 1% Sulfobetaine SB12 (Z3-12) by
20   sonicating using a VibraCell tuner with a microtip
for 3 X 30 seconds. This was performed in a crushed
ice/salt mix for cooling. Following sonication, the
mixture was slowly mixed for 30 minutes at 4°C to
finish solubilizing membrane bound proteins. This
25   mixture was centrifuged in a Beckman ultracentrifuge
using a type TI45 rotor at 36,000 rpm for 45 min-
utes. The supernatant was diluted with Lysis Buffer
containing 10 μg/mL calpain inhibitors I and II.
The precipitated protein was centrifuged for 20
30   minutes at 9,000 rpm in a Beckman JA-10 rotor. The
recovered supernatant then was subjected to Mimetic
Blue® AP Agarose Chromatography.

- 82 -

To run the Mimetic Blue® AP Agarose Col-
umn, the resin initially was shielded by the appli-
cation of 10 bed volumes of 1% polyvinylpyrrolidone
(i.e., MW of 40,000) to block nonspecific binding

5   sites. The loosely bound PVP-40 was removed by
washing with 10 bed volumes of 2 M NaCl, and 10 mM
sodium citrate pH 3.4. Just prior to addition of
the solubilized PCE1C3 sample, the column was equil-
ibrated with 5 bed volumes of Column Buffer A (50 mM

10   MOPS pH 7.4, 10 μM $ZnSO_4$, 5 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1
mM DTT, 2 mM benzamidine HCl).

The solubilized sample was applied to the
column at a flow rate of 2 mL/min with recycling
such that the total sample was applied 4 to 5 times

15   in 12 hours. After loading was completed, the col-
umn was washed with 10 column volumes of Column
Buffer A, followed by 5 column volumes of Column
Buffer B (Column Buffer A containing 20 mM 5'-AMP),
and followed by 5 column volumes of Column Buffer C

20   (50 mM MOPS pH 7.4, 10 μM $ZnSO_4$, 0.1 mM $CaCl_2$, 1 mM
DTT, and 2 mM benzamidine HCl). The enzyme was
eluted into three successive pools. The first pool
consisted of enzyme from a 5-bed volume wash with
Column Buffer C containing 1 mM cAMP. The second

25   pool consisted of enzyme from a 10-bed volume wash
with Column Buffer C containing 1 M NaCl. The final
pool of enzyme consisted of a 5-bed volume wash with
Column Buffer C containing 1 M NaCl and 20 mM cAMP.

The active pools of enzyme were collected

30   and the cyclic nucleotide removed via conventional
gel filtration chromatography or chromatography on
hydroxyapatite resins. Following removal of cyclic
nucleotides, the enzyme pools were dialyzed against

Dialysis Buffer containing 25 mM MOPS pH 7.4, 10 μM
$ZnSO_4$, 500 mM NaCl, 1 mM $CaCl_2$, 1 mM DTT, 1 mM
benzamidine HCl, followed by dialysis against Dialy-
sis buffer containing 50% glycerol. The enzyme was

5   quick-frozen with the aid of dry ice and stored at
-70°C.

The resultant preparations were about >90%
pure by SDS-PAGE. These preparations had specific
activities of about 0.1 to 1.0 μmol cAMP hydrolyzed

10   per minute per milligram protein.

Purification of PDE2 from *S. cerevisiae*

Frozen yeast cell pellets from strain YI34

15   (10g, stored at -70°C) were allowed to thaw on ice
in 25 mL of Lysis Buffer (50 mM MOPS, pH 7.2, 1 mM
EDTA, 1 mM EGTA, 0.1 mM DTT, 0.1 mM 4-(2-amino-
ethyl)benzenesulfonyl fluoride (AEBSF), 1 μg/mL of
pepstatin, leupeptin, aprotinin, calpain inhibitors

20   I and II, and 2 mM benzamidine). Cells were lysed
by three passages through a French® pressure cell
(SLM-Aminco®, Spectronic Instruments). The lysate
was centrifuged at 36,000 rpm in a Beckman Ultracen-
trifuge rotor Type 45Ti for 60 minutes at 4°C. The

25   supernatant was separated from sediment and passed
through a 15 mL Epoxy-cGMP Sepharos® resin at 4°C
two times at about 0.5 mL/min. The column subse-
quently was washed with 45 mL of Wash Buffer 1 (50
mM MOPS, pH 7.2, 0.1 mM EDTA, 0.1 mM DTT). Follow-

30   ing this wash, the column was washed with 45 mL of
Wash Buffer 2 (Wash Buffer 1 containing 0.5 M NaCl).
Following this salt wash, the column was washed with
15 mL of Wash Buffer 3 (Wash Buffer 1 containing

0.25 M NaCl). The column was transferred to room
temperature and allowed to warm. Approximately 25
mL of Elution Buffer (Wash Buffer 3 containing 10 mM
cGMP, maintained at room temperature) was applied to
5   the column and the effluent was collected in 2 mL
fractions. Small aliquots of each of the fractions
were diluted 20-fold in PBS containing 5 mM $MgCl_2$ to
allow hydrolysis of the competing ligand and to aid
detection of PDE2 activity. Active fractions were
10   passed through a Pharmacia PD-10® gel filtration
column to exchange into Wash Buffer 3. This ex-
changed pool was diluted 50% v/v with sterile 80%
glycerol and stored at -20°C. The resultant prepa-
rations were greater than 85% pure as judged by SDS-
15   PAGE with subsequent staining of protein by
Coomassie R-250. These preparations had specific
activities of about 150 to 250 μmol cGMP hydrolyzed
per minute per milligram protein.

20   **Preparation of PDE3A from Sf9 Cells**

Cells (2 X 1010) were suspended in Lysis
Buffer containing 50 mM MOPS pH 7.5, 2 mM DTT, 2 mM
benzamidine HCl, 5 μM $ZnSO_4$, 0.1 mM $CaCl_2$, 20 μg/mL
25   calpain inhibitors I and II, and 5 μg/mL each of
leupeptin, pepstatin, and aprotinin. The mixture
was sonicated twice for 30 seconds and the cells
were lysed in a French® pressure cell (SLM-Aminco®,
Spectronic Instruments) at 4°C. The lysate was
30   centrifuged 100,000 X g for 45 minutes. The pellet
was washed once in Lysis Buffer and suspended in 46
mL Lysis Buffer with a Dounce homogenizer. Aliquots
were stored at -70°C. These preparations had spe-

- 85 -

cific activities of about 1 to 2 nmol cAMP hydro-
lyzed per minute per milligram protein.

Human PDE4A, 4B, 4C, 4D Preparations

5

Preparation of PDE4A from *S. cerevisiae*

Yeast cells (50 g of yeast strain YI26
harboring HDUN1.46) were thawed at room temperature
10   by mixing with 50 mL of Lysis Buffer (50 mM MOPS pH
7.5, 10 μM ZnSO$_4$, 2 mM MgCl$_2$, 14.2 mM 2-mercapto-
ethanol, 5 μg/mL each of pepstatin, leupeptin,
aprotinin, 20 μg/mL each of calpain inhibitors I and
II, and 2 mM benzamidine HCl). Cells were lysed in
15   a French® pressure cell (SLM-Aminco®, Spectronic
Instruments) at 10°C. The extract was centrifuged
in a Beckman JA-10 rotor at 9,000 rpm for 22 minutes
at 4°C. The supernatant was removed and centrifuged
in a Beckman TI45 rotor at 36,000 rpm for 45 minutes
20   at 4°C.
PDE4A was precipitated from the high-speed
supernatant by the addition of solid ammonium sul-
fate (0.26 g/mL supernatant) while stirring in an
ice bath and maintaining the pH between 7.0 and 7.5.
25   The precipitated proteins containing PDE4A were
collected via centrifugation in a Beckman JA-10
rotor at 9,000 rpm for 22 minutes. The precipitate
was resuspended in 50 mL of Buffer G (50 mM MOPS pH
7.5, 10 μM ZnSO$_4$, 5 mM MgCl$_2$, 100 mM NaCl, 14.2 mM 2-
30   mercaptoethanol, 2 mM benzamidine HCl, 5 μg/mL each
of leupeptin, pepstatin, and aprotinin, and 20 μg/mL
each of calpain inhibitors I and II) and passed
through a 0.45 μm filter.

- 86 -

The resuspended sample (50 to 100 mL) was loaded onto a 5 X 100 cm column of Pharmacia SEPHACRYL® S-300 equilibrated in Buffer G. Enzyme activity was eluted at a flow rate of 2 mL/min and
5   pooled for later fractionation.

The PDE4A isolated from gel filtration chromatography was applied to a 1.6 X 20 cm column of Sigma Cibacron Blue Agarose-type 300 (10 mL) equilibrated in Buffer A (50 mM MOPS pH 7.5, 10 μM
10   $ZnSO_4$, 5 mM $MgCl_2$, 14.2 mM 2-mercaptoethanol, and 100 mM benzamidine HCl). The column was washed in succession with 50 to 100 mL of Buffer A, 20 to 30 mL of Buffer A containing 20 mM 5'-AMP, 50 to 100 mL of Buffer A containing 1.5 M NaCl, and 10 to 20 mL of
15   Buffer C (50 mM Tris HCl pH 8, 10 μM $ZnSO_4$, 14.2 mM 2-mercaptoethanol, and 2 mM benzamidine HCl). The enzyme was eluted with 20 to 30 mL of Buffer C containing 20 mM cAMP.

The PDE activity peak was pooled, and
20   precipitated with ammonium sulfate (0.33 g/mL enzyme pool) to remove excess cyclic nucleotide. The precipitated proteins were resuspended in Buffer X (25 mM MOPS pH 7.5, 5 μM $ZnSO_4$, 50 mM NaCl, 1 mM DTT, and 1 mM benzamidine HCl), and desalted via gel
25   filtration on a Pharmacia PD-10® column per manufacturer's instructions. The enzyme was quick-frozen in a dry ice/ethanol bath and stored at -70°C.

The resultant preparations were about >80% pure by SDS-PAGE. These preparations had specific
30   activities of about 10 to 40 μmol cAMP hydrolyzed per minute per milligram protein.

- 87 -

Preparation of PDE4B from *S. cerevisiae*

Yeast cells (150 g of yeast strain YI23 harboring HDUN2.32) were thawed by mixing with 100 mL glass beads (0.5 mM, acid washed) and 150 mL Lysis Buffer (50 mM MOPS pH 7.2, 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, 5 μg/mL each of pepstatin, leupeptin, aprotinin, calpain inhibitors I and II) at room temperature. The mixture was cooled to 4°C, transferred to a Bead-Beater®, and the cells lysed by rapid mixing for 6 cycles of 30 seconds each. The homogenate was centrifuged for 22 minutes in a Beckman J2-21M centrifuge using a JA-10 rotor at 9,000 rpm and 4°C. The supernatant was recovered and centrifuged in a Beckman XL-80 ultracentrifuge using a TI45 rotor at 36,000 rpm for 45 minutes at 4°C. The supernatant was recovered and PDE4B was precipitated by the addition of solid ammonium sulfate (0.26 g/mL supernatant) while stirring in an ice bath and maintaining the pH between 7.0 and 7.5. This mixture was then centrifuged for 22 minutes in a Beckman J2 centrifuge using a JA-10 rotor at 9,000 rpm (12,000 X g). The supernatant was discarded and the pellet was dissolved in 200 mL of Buffer A (50 mM MOPS pH 7.5, 5 mM MgCl$_2$, 1 mM DTT, 1 mM benzamidine HCl, and 5 μg/mL each of leupeptin, pepstatin, and aprotinin). The pH and conductivity were corrected to 7.5 and 15-20 mS, respectively.

The resuspended sample was loaded onto a 1.6 X 200 cm column (25 mL) of Sigma Cibacron Blue Agarose-type 300 equilibrated in Buffer A. The sample was cycled through the column 4 to 6 times over the course of 12 hours. The column was washed

- 88 -

in succession with 125 to 250 mL of Buffer A, 125 to
250 mL of Buffer A containing 1.5 M NaCl, and 25 to
50 mL of Buffer A. The enzyme was eluted with 50 to
75 mL of Buffer E (50 mM Tris HCl pH 8, 2 mM EDTA, 2
5   mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, and 20 mM
cAMP) and 50 to 75 mL of Buffer E containing 1 M
NaCl. The PDE activity peak was pooled, and precip-
itated with ammonium sulfate (0.4 g/mL enzyme pool)
to remove excess cyclic nucleotide. The precipi-
10   tated proteins were resuspended in Buffer X (25 mM
MOPS pH 7.5, 5 μM $ZnSO_4$, 50 mM NaCl, 1 mM DTT, and 1
mM benzamidine HCl) and desalted via gel filtration
on a Pharmacia PD-10® column per manufacturer's
instructions. The enzyme pool was dialyzed over-
15   night against Buffer X containing 50% glycerol.
This enzyme was quick-frozen in a dry ice/ethanol
bath and stored at -70°C.

        The resultant preparations were about >90%
pure by SDS-PAGE. These preparations had specific
20   activities of about 10 to 50 μmol cAMP hydrolyzed
per minute per milligram protein.

Preparation of PDE4C from *S. cerevisiae*

25   Yeast cells (150 g of yeast strain YI30
harboring HDUN3.48) were thawed by mixing with 100
mL glass beads (0.5 mM, acid washed) and 150 mL
Lysis Buffer (50 mM MOPS pH 7.2, 2 mM EDTA, 2 mM
EGTA, 1 mM DTT, 2 mM benzamidine HCl, 5 μg/mL each
30   of pepstatin, leupeptin, aprotinin, calpain inhibi-
tors I and II) at room temperature. The mixture was
cooled to 4°C, transferred to a BEAD-BEATER®, and
the cells lysed by rapid mixing for 6 cycles of 30

- 89 -

sec each. The homogenate was centrifuged for 22
minutes in a Beckman J2-21M centrifuge using a JA-10
rotor at 9,000 rpm and 4°C. The supernatant was
recovered and centrifuged in a Beckman XL-80 ultra-
5   centrifuge using a TI45 rotor at 36,000 rpm for 45
minutes at 4°C.

The supernatant was recovered and PDE4C
was precipitated by the addition of solid ammonium
sulfate (0.26 g/mL supernatant) while stirring in an
10   ice bath and maintaining the pH between 7.0 and 7.5.
Thirty minutes later, this mixture was centrifuged
for 22 minutes in a Beckman J2 centrifuge using a
JA-10 rotor at 9,000 rpm (12,000 X g). The super-
natant was discarded and the pellet was dissolved in
15   200 mL of Buffer A (50 mM MOPS pH 7.5, 5 mM MgCl$_2$, 1
mM DTT, 2 mM benzamidine HCl, and 5 μg/mL each of
leupeptin, pepstatin, and aprotinin). The pH and
conductivity were corrected to 7.5 and 15-20 mS,
respectively.

20   The resuspended sample was loaded onto a
1.6 X 20 cm column (25 mL) of Sigma Cibacron Blue
Agarose-type 300 equilibrated in Buffer A. The
sample was cycled through the column 4 to 6 times
over the course of 12 hours. The column was washed
25   in succession with 125 to 250 mL of Buffer A, 125 to
250 mL of Buffer A containing 1.5 M NaCl, and then
25 to 50 mL of Buffer A. The enzyme was eluted with
50 to 75 mL of Buffer E (50 mM Tris HCl pH 8, 2 mM
EDTA, 2 mM EGTA, 1 mM DTT, 2 mM benzamidine HCl, and
30   20 mM cAMP) and 50 to 75 mL of Buffer E containing 1
M NaCl. The PDE4C activity peak was pooled, and
precipitated with ammonium sulfate (0.4 g/mL enzyme
pool) to remove excess cyclic nucleotide. The pre-

- 90 -

cipitated proteins were resuspended in Buffer X (25
mM MOPS pH 7.2, 5 μM ZnSO$_4$, 50 mM NaCl, 1 mM DTT,
and 1 mM benzamidine HCl) and desalted via gel fil-
tration on a Pharmacia PD-10® column per manufac-
5   turer's instructions. The enzyme pool was dialyzed
overnight against Buffer X containing 50% glycerol.
This enzyme was quick-frozen in a dry ice/ethanol
bath and stored at -70°C.

        The resultant preparations were about >80%
10   pure by SDS-PAGE. These preparations had specific
activities of about 10 to 20 μmol cAMP hydrolyzed
per minute per milligram protein.

Preparation of PDE4D from _S. cerevisiae_

15

        Yeast cells (100 g of yeast strain YI29
harboring HDUN4.11) were thawed by mixing with 150
mL glass beads (0.5 mM, acid washed) and 150 mL
Lysis Buffer (50 mM MOPS pH 7.2, 10 μM ZnSO$_4$, 2 mM
20   MgCl$_2$, 14.2 mM 2-mercaptoethanol, 2 mM benzamidine
HCl, 5 μg/mL each of pepstatin, leupeptin, aprotin-
in, calpain inhibitors I and II) at room tempera-
ture. The mixture was cooled to 4°C, transferred to
a Bead-Beater®, and the cells lysed by rapid mixing
25   for 6 cycles of 30 sec each. The homogenate was
centrifuged for 22 minutes in a Beckman J2-21M cen-
trifuge using a JA-10 rotor at 9,000 rpm and 4°C.
The supernatant was recovered and centrifuged in a
Beckman XL-80 ultracentrifuge using a TI45 rotor at
30   36,000 rpm for 45 minutes at 4°C. The supernatant
was recovered and PDE4D was precipitated by the
addition of solid ammonium sulfate (0.33 g/mL super-
natant) while stirring in an ice bath and maintain-

- 91 -

ing the pH between 7.0 and 7.5. Thirty minutes
later, this mixture was centrifuged for 22 minutes
in a Beckman J2 centrifuge using a JA-10 rotor at
9,000 rpm (12,000 X g). The supernatant was dis-

5   carded and the pellet was dissolved in 100 mL of
Buffer A (50 mM MOPS pH 7.5, 10 μM $ZnSO_4$, 5 mM $MgCl_2$,
14.2 mM 2-mercaptoethanol, 100 mM benzamidine HCl,
and 5 μg/mL each of leupeptin, pepstatin, aprotinin,
calpain inhibitor I and II). The pH and conductiv-

10   ity were corrected to 7.5 and 15-20 mS, respec-
tively.

At a flow rate of 0.67 mL/min, the resus-
pended sample was loaded onto a 1.6 X 20 cm column
(10 mL) of Sigma Cibacron Blue Agarose-type 300

15   equilibrated in Buffer A. The column was washed in
succession with 50 to 100 mL of Buffer A, 20 to 30
mL of Buffer A containing 20 mM 5'-AMP, 50 to 100 mL
of Buffer A containing 1.5 M NaCl, and then 10 to 20
mL of Buffer C (50 mM Tris HCl pH 8, 10 μM $ZnSO_4$,

20   14.2 mM 2-mercaptoethanol, 2 mM benzamidine HCl).
The enzyme was eluted with 20 to 30 mL of Buffer C
containing 20 mM cAMP.

The PDE4D activity peak was pooled and
precipitated with ammonium sulfate (0.4 g/mL enzyme

25   pool) to remove excess cyclic nucleotide. The pre-
cipitated proteins were resuspended in Buffer X (25
mM MOPS pH 7.2, 5 μM $ZnSO_4$, 50 mM NaCl, 1 mM DTT,
and 1 mM benzamidine HCl) and desalted via gel fil-
tration on a Pharmacia PD-10® column per manufac-

30   turer's instructions. The enzyme pool was dialyzed
overnight against Buffer X containing 50% glycerol.
This enzyme preparation was quick-frozen in a dry
ice/ethanol bath and stored at -70°C.

The resultant preparations were about >80%
pure by SDS-PAGE. These preparations had specific
activities of about 20 to 50 μmol cAMP hydrolyzed
per minute per milligram protein.

5

Purification of PDE5 from *S. cerevisiae*

Cell pellets (29 g) were thawed on ice
with an equal volume of Lysis Buffer (25 mM Tris

10   HCl, pH 8, 5 mM MgCl$_2$, 0.25 mM DTT, 1 mM benzamidine,
and 10 μM ZnSO$_4$). Cells were lysed in a Micro-
fluidizer® (Microfluidics Corp.) using nitrogen at
20,000 psi. The lysate was centrifuged and filtered
through 0.45 μm disposable filters. The filtrate

15   was applied to a 150 mL column of Q SEPHAROSE® Fast-
Flow (Pharmacia). The column was washed with 1.5
volumes of Buffer A (20 mM Bis-Tris Propane, pH 6.8,
1 mM MgCl$_2$, 0.25 mM DTT, 10 μM ZnSO$_4$) and eluted with
a step gradient of 125 mM NaCl in Buffer A followed

20   by a linear gradient of 125-1000 mM NaCl in Buffer
A. Active fractions from the linear gradient were
applied to a 180 mL hydroxyapatite column in Buffer
B (20 mM Bis-Tris Propane (pH 6.8), 1 mM MgCl$_2$, 0.25
mM DTT, 10 μM ZnSO$_4$, and 250 mM KCl). After load-

25   ing, the column was washed with 2 volumes of Buffer
B and eluted with a linear gradient of 0-125 mM
potassium phosphate in Buffer B. Active fractions
were pooled, precipitated with 60% ammonium sulfate,
and resuspended in Buffer C (20 mM Bis-Tris Propane,

30   pH 6.8, 125 mM NaCl, 0.5 mM DTT, and 10 μM ZnSO$_4$).
The pool was applied to a 140 mL column of
SEPHACRYL® S-300 HR and eluted with Buffer C. Ac-

- 93 -

tive fractions were diluted to 50% glycerol and stored at -20°C.

The resultant preparations were about 85% pure by SDS-PAGE. These preparations had specific
5   activities of about 3 μmol cGMP hydrolyzed per minute per milligram protein.

Preparation of PDE7 from *S. cerevisiae*

10   Cell pellets (126 g) were thawed and resuspended at room temperature for about 30 minutes with an equal volume of Lysis Buffer (50 mM Tris HCl, pH 8, 1 mM EDTA, 1 mM DTT, 50 mM NaCl, 2 mM benzamidine HCl, and 5 μg/mL each of pepstatin,
15 leupeptin, and aprotinin). The cells were lysed at 0-4°C with the aid of glass beads (125 mL) in a Bead-Beater® for 6 X 30 second cycles. The lysate was centrifuged and filtered through 0.45 μm disposable filters. The filtered extract (178 mL) was
20 distributed into 4 mL aliquots, quick-frozen with dry ice, and stored in a freezer at -70°C. These preparations were stable to several cycles of freezing and thawing and had specific activities of about 50 to 100 pmol cAMP hydrolyzed per minute per milli-
25 gram protein.

Lipopolysaccharide-Stimulated TNFα Release from Human Peripheral Blood Lymphocytes

30
          To assess the ability of a compound to reduce TNFα secretion in human peripheral blood lymphocytes (PBL), the following tests were performed. Previous studies have demonstrated that

- 94 -

incubation of human PBL with cAMP-elevating agents,
such as prostaglandin E21 forskolin, 8-bromo-cAMP,
or dibutryl-cAMP, inhibits the secretion of TNFα by
the cells when stimulated by lipopolysaccharide

5   (LPS; endotoxin). Accordingly, preliminary experi-
ments have been performed to demonstrate that selec-
tive PDE4 inhibitors, such as rolipram, inhibit LPS-
induced TNFα secretion from human lymphocytes in a
dose-dependent fashion. Hence, TNFα secretion from

10   human PBL was used as a standard for the ability of
a compound to elevate intracellular cAMP concentra-
tions and/or to inhibit PDE4 activity within the
cell.

         Heparinized blood (approximately 30 mL)

15   drawn from human volunteers was mixed 1:1 with
Dulbecco's modified phosphate-buffered saline. This
mixture was mixed 1:1 with HISTOPAQUE® and centri-
fuged at 1,500 rpm at room temperature without brak-
ing in the swinging bucket of a Beckman model TJ6

20   centrifuge. Erythrocytes were centrifuged to the
bottom of the tubes, and serum remained at the sur-
face of the tubes. A layer containing lymphocytes
sedimented between the serum and HISTOPAQUE® layers,
and was removed by aspiration to a fresh tube. The

25   cells were quantified and adjusted to 3 X 10^6
cells/mL and a 100 μL aliquot is placed into the
wells of a 96 well plate. Test compounds and RPMI
media (Gibco/BRL Life Sciences) are added to each of
the wells 15 minutes prior to addition of bacterial

30   LPS (25 mg/mL). The mixture was allowed to incubate
for 20 hours at 37°C in a humidified chamber. The
cells then were separated by centrifuging at 800 rpm
for 5 minutes at room temperature. An aliquot of

- 95 -

180 μL of supernatant was transferred to a new plate
for determination of TNFα concentration. TNFα pro-
tein in the cell supernatant fluids was measured
using a commercially available enzyme-linked immuno-
5   sorbent assay (ELISA) (CYTOSCREEN® Immunoassay Kit
from Biosource International).

The cell-based assay provided the follow-
ing results for various compounds of the present
invention. The EC$_{50}$ values (i.e., effective concen-
10 tration of the compound capable of inhibiting 50% of
the total TNFα) illustrate the ability of the pres-
ent compounds to inhibit LPS-stimulated TNFα release
from human PBL.

The following summarizes the IC$_{50}$ values
15 determined for compounds of structural formulae (I)
and (II) against human recombinant PDE4. In the
following table, R^3 is hydrogen and n is 1, unless
otherwise noted.

20

25

30

35

(I)
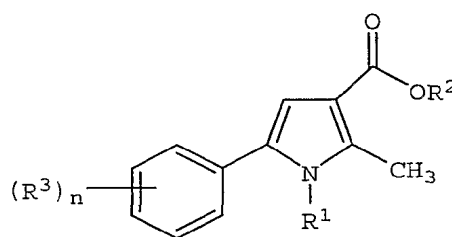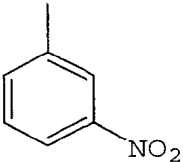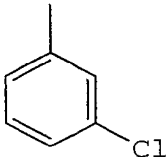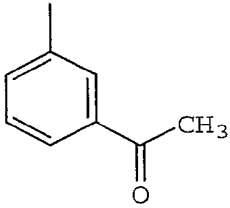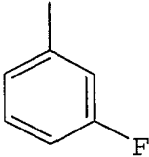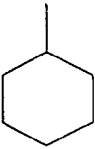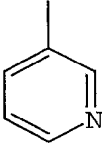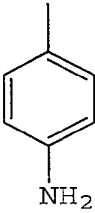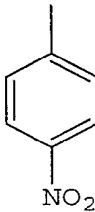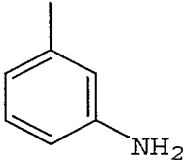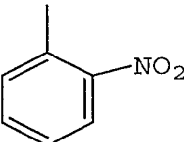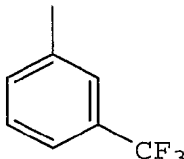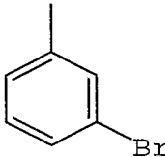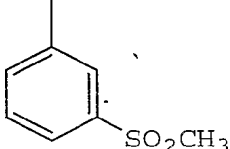
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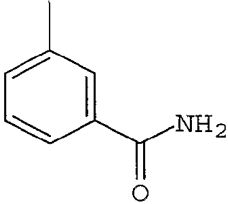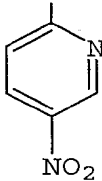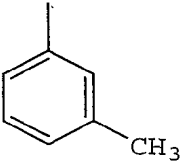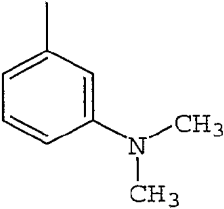
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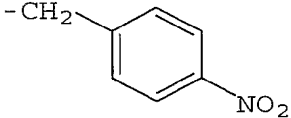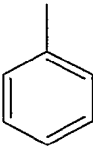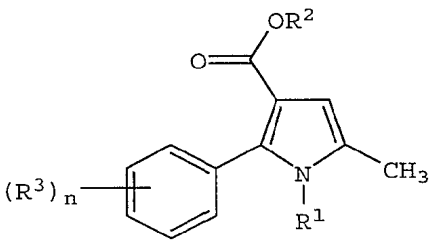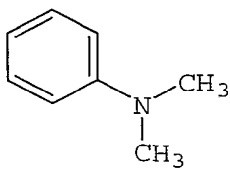
Example	R^1	R^2	PDE4 IC$_{50}$ (μM)
22	C$_6$H$_4$NO$_2$	Et	0.15
3	C$_6$H$_4$Cl	Et	0.20
8	C$_8$H$_7$O	Et	0.59
18	C$_6$H$_4$F	Et	0.62

Example	R^1	R^2	PDE4 IC_{50} (μM)
20	 C_6H_{11}	Et	0.99
19	 C_3H_4N	Et	1.28
38	 NH_2 C_6H_6N	Et	1.30
33	 NO_2 $C_6H_4NO_2$	Et	1.40
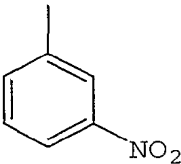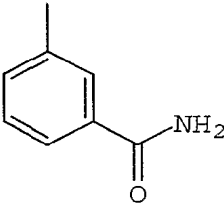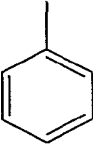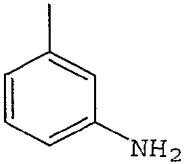
Example	R^1	R^2	PDE4 IC$_{50}$ (μM)
41	![structure] C_6H_6N	Et	1.42
34	![structure] $C_6H_4NO_2$	Et	1.65
28	![structure] $C_7H_4F_3$	Et	2.28
26	![structure] C_6H_4Br	Et	3.07
10	![structure] $C_7H_7O_2S$	Et	4.38

5

Example	R^1	R^2	PDE4 IC_{50} (μM)
11	C_7H_6NO	Et	5.45
35	$C_5H_3N_2O_2$	Et	5.74
5	C_7H_7	Et	6.10
1	$C_8H_{10}N$	Et	8.22

Example	R^1	R^2	PDE4 IC$_{50}$ (μM)
30	$-CH_2-$\ (4-nitrobenzyl structure)\ $C_7H_5N_2O_4$	Et	13.42
44	(phenyl structure)\ C_6H_5	H	54.16

$$\text{(II)}$$

Et=ethyl

H=hydrogen

| 13 | (N,N-dimethylaniline structure)\
 $C_8H_{10}N$ | Et | 0.15 |
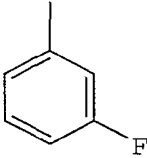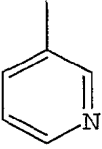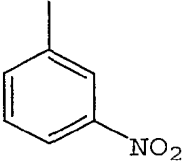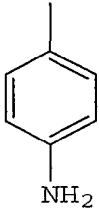
Example	R^1	R^2	PDE4 IC$_{50}$ (μM)
39	$C_6H_4NO_2$	Et	0.20
12	C_7H_6NO	Et	0.22
21	C_6H_5	Et	0.59
40	C_6H_6N	Et	1.29

Example	R^1	R^2	PDE4 IC$_{50}$ (μM)
42	 C$_6$H$_3$Cl$_2$	Et	1.49
4	 C$_7$H$_7$	Et	2.90
9	 C$_7$H$_7$O$_2$S	Et	4.58
2	 C$_6$H$_4$Cl	Et	5.45
29	 C$_7$H$_4$F$_3$	Et	6.78

Example	R^1	R^2	PDE4 IC_{50} (μM)
7	C_8H_7O	Et	6.83
31	$C_7H_6NO_2$	Et	5.13
6	C_6H_4Br	Et	8.55
46	$C_9H_9O_2$	Et	8.70
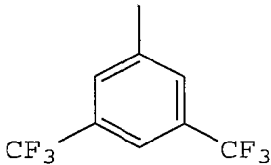
- 104 -

Example	R^1	R^2	PDE4 IC$_{50}$ (μM)
17	C$_6$H$_4$F	Et	13.21
32	C$_5$H$_4$N	Et	13.39
43	C$_6$H$_4$NO$_2$	H	15.63
37	C$_6$H$_6$N	Et	16.00

Example	R¹	R²	PDE4 IC$_{50}$ (μM)
24	$C_7H_5O_2$	Et	16.53
21	C_6H_5	Et	31.29
36	$C_6H_4NO_2$	Et	110.00
23	$C_5H_3N_2O_2$	Et	133.01

- 106 -

Example	R¹	R²	PDE4 IC$_{50}$ (μM)
45	 $C_8H_3F_6$	Et	193.71

5   The data presented above shows that com-
pounds of formula (I) and (II) are potent and selec-
tive inhibitors of PDE4, e.g., the compounds have an
IC$_{50}$ vs. human recombinant PDE4 of about 0.15 to
about 195 μm. As an added advantage, the compounds
10   of formula (I) and (II) reduced or eliminated the
adverse side effects, such as CNS effects, associ-
ated with prior PDE4 inhibitors. In particular,
compounds of formulae (I) and (II) were tested in
cell-based assays and in animal models to illustrate
15   the efficacy of the compounds with respect to inhib-
iting PDE4 both *in vitro* and *in vivo*.

          The cell-based assay provided the follow-
ing results for various pyrrole compounds of the
present invention. The EC$_{50}$ values (i.e., effective
20   concentration of the compound capable of inhibiting
50% of the total TNFα) illustrate the ability of the
present compounds to inhibit LPS-stimulated TNFα
release from human peripheral blood lymphocytes.

Compound	PDE4 IC_{50} (μm)	PBL/TNFα EC_{50} (μM)
Ex. 22	.15	.87
Ex. 3	.20	1.50
Ex. 21	.40	10.00
Ex. 40	1.29	6.72
Ex. 42	1.49	100.0

The above Table and FIG. 1 illustrate the ability of compounds of formulae (I) and (II) to inhibit PDE4 activity and TNFα release. Preferred compounds have a PBL/TNFα EC_{50} about 500 nM or less, and preferably about 200 nM or less. More preferred compounds have a PBL/TNFα EC_{50} of about 100 nM or less.

To achieve the full advantages of the present invention, the compound has an IC_{50} vs. human recombinant PDE4 of about 100 nM or less and a PBL/TNFα EC_{50} of about 500 nM or less. More preferably, the compound has an IC_{50} of about 50 nM or less and a PBL/TNFα EC_{50} of about 100 nM or less.

- 108 -

Animal Models

Assay for Inhibition of
Serum TNFα Levels in Mammals
5   (Mouse/TNFα ED_{50} (mg/kg))

          In order to assess the ability of a com-
     pound to reduce serum TNFα levels in mammals, the
10   following protocol was employed. Those skilled in
     the art appreciate that previous studies have demon-
     strated that incubation of LPS-activated human
     monocytes with agents that can elevate cAMP, like
     PGE2, forskolin, and the dbcAMP, inhibited secretion
15   of TNFα. PDE4 inhibitors like rolipram, which also
     elevate cAMP, have been found to inhibit serum TNFα
     as well. Rolipram has also been found to inhibit
     secretion of TNFα from LPS-activated mouse macro-
     phages. Accordingly, in vivo efficacy of a PDE4
20   reducing compound was shown by dosing with compound
     and measuring reduction of serum TNFα levels in LPS-
     injected mice. Female C3H mice, 20-25 gm body
     weight, were fasted overnight and dosed intraperi-
     toneally with test compound in appropriate vehicle
25   60 minutes before LPS injection. Five μg of LPS was
     then injected intraperitoneally into the mice.
     Ninety minutes after LPS injection, mice were bled
     from the heart. Blood was allowed to clot overnight
     at 4°C. Samples were centrifuged for 10 minutes in
30   a microcentrifuge and the serum removed and stored
     at -20°C until analysis. Serum levels of TNFα were
     subsequently measured using a commercially available
     ELISA kit (Genzyme) following the protocol enclosed
     in the kit. The inhibition of serum TNFα levels

- 109 -

caused by the compound was determined relative to serum TNFα levels in control mice receiving vehicle alone. The results are summarized in the plots of FIG. 1.

**Combined Mouse endotoxin-stimulated TNFα
Release and Locomotor Activity Assay**

The purpose of this study was to determine the efficacy of PDE4 inhibitors *in vivo* in an LPS mouse model together with a determination with re-spect to central nervous system (CNS) side-effects manifested by a decrease in spontaneous mobility.

The test animals were female Balb/c mice, having an average weight of about 20 g. The PDE4 inhibitors, formulated in 30% Cremophor® EL, were administered via intraperitoneal (i.p.) injections at doses of 0.1, 1.0, 10.0, and 100 mg/kg. Individ-ual dose volumes (about 150 μL) were adjusted based on the body weights measured. One hour later, 5 mg/kg LPS in a final volume of 200 μL was injected via the tail vein to each animal. Ninety minutes following the LPS treatment, the animals were bled and serum samples were collected before being stored at -70°C until assayed.

For efficacy determination, the serum samples were diluted two-fold and TNFα levels were determined using the CYTOSCREEN® Immunoassay Kit (Biosource International). The data were averaged between triplicate sample subjects for each of the tested compounds.

- 110 -

For side-effect profiling, a subjective visual scoring system was utilized at 5min and 20min after administration of PDE4 inhibitors. Vehicle control animals were rated a single "+" and animals that were effectively immobilized and stretched out on the bottom of the cage with little detectable movement were rated as "++++." Alternatively, a semi-automated "open field" system (e.g., a Photo-beam Activity Measurement System as sold by San Diego Instruments) for monitoring movements was used for assessing the effect of PDE4 inhibitors on mice and/or rats. In this instance, the subjects could be monitored continuously over pre-set intervals.

Movement of the X-Y plane, or rearing up on the hind legs, was quantified by counting the number of "light-beam" crosses per unit of time. A decrease in the number of activity events is di-rectly proportional to the mobility or immobiliza-tion of the animal. The quantitative scoring corre-lated well with the subjective measurements de-scribed above.

Compound	Vehicle control	1 mg/kg	10 mg/kg	100 mg/kg
Comparative Example[1]		3	3	3
Example 22	3	3	3	3

[1] _trans_-3-(3-cyclopentoxy-4-methoxyphenyl)-1-methoxycarbonyl-4-methyl-4-(methylcarbonyl)pyrrolidine, i.e., Example 12 of Feldman et al. U.S. Patent No. 5,665,754, incorporated herein by reference.

- 111 -

Compound No.	Doses (mg/kg)	Sedative Effect	%TNFα Inhibition
Comparative Example[1]	1	--	43
	10	+	100
	100	+++	100
Example 22	1	--	13
	10	--	84
	100	--	100

The data presented above show that compounds of formulae (I) and (II) are potent and selective inhibitors of PDE4. As an important added advantage, the compounds of formulae (I) and (II) also reduced or eliminated the adverse CNS side effects associated with prior PDE4 inhibitors. Compounds of formulae (I) and (II) were further tested for emetogenic properties in animal models to further illustrate the efficacy of the compounds. The method and results of the emetogenic test are set forth below.

The compounds of the present invention are useful for selectively inhibiting PDE4 activity in a mammal, without exhibiting the adverse CNS and emetic effects associated with prior PDE4 inhibitors.
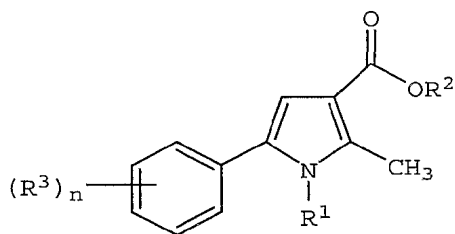
Obviously, many modifications and variations of the invention as hereinbefore set forth can be made without departing from the spirit and scope thereof and, therefore, only such limitations should be imposed as are indicated by the appended claims.
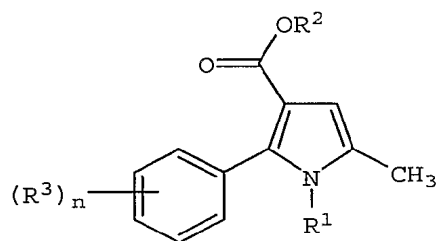
WHAT IS CLAIMED IS:

1.   A compound having a formula

or

wherein R^1 is selected from the group consisting of optionally substituted alkyl, cyclo-alkyl, aryl, heteroaryl, alkaryl, aralkyl, hetero-aralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, hetero-aryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)$alkyl, NR^4R^5, $C(=O)NR^5R^6$, $C(=O)O$alkyl, CO_2H, $OC(=O)$alkyl, nitro, halo, alkylthio, SO_2(alkyl), SO_3H, and haloalkyl;

- 113 -

R^4 and R^5, independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3,

with the proviso that when R^2 is ethyl, R^1 is different from 3-nitrophenyl, 2,3-dichlorophenyl, and 3,5-bis-trifluoromethylphenyl.

2.   The compound of claim 1 wherein R^1 is selected from the group consisting of aryl, hetero-aryl, heteroalkaryl, alkaryl, and cycloalkyl.
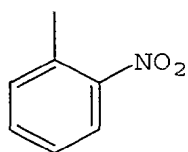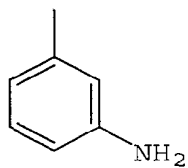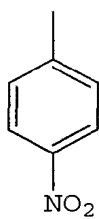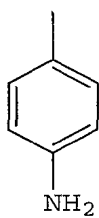

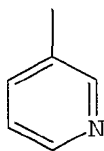3.   The composition of claim 1 wherein R^1 is selected from the group consisting of aryl, heteroaryl, cycloalkyl, and alkaryl, optionally substituted with one or more of NR^4R^5, halo, alkyl, $C(=O)$alkyl, SO_2(alkyl), $C(=O)NR^5R^6$, SO_3H, NO_2, CO_2H, $C(=O)O$alkyl, and CF_3.

- 114 -

4. The compound of claim 1 wherein R¹ is selected from the group consisting of:

- 115 -
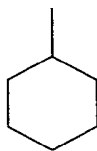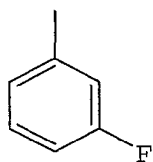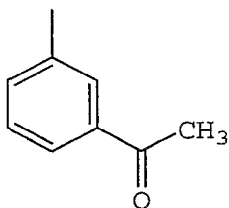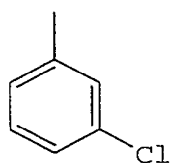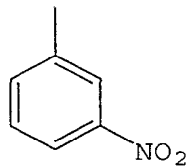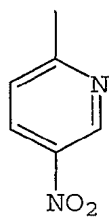
- 117 -
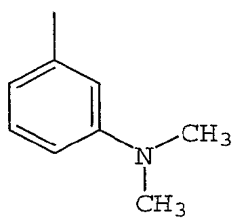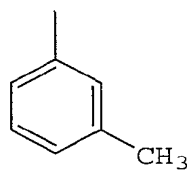
- 118 -
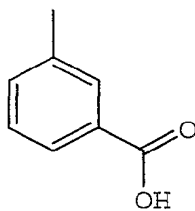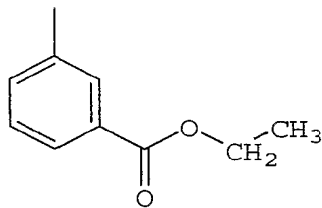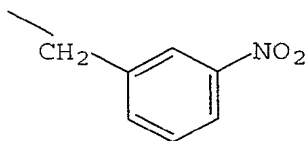
and
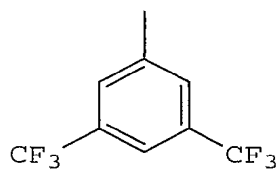
5.　The composition of claim 1 wherein R^2 is selected from the group consisting of hydrogen and lower alkyl.

- 119 -

6.   The composition of claim 1 wherein R^1 is phenyl, pyridyl, or cyclohexyl, optionally substituted; R^2 is hydrogen or $C_{1\text{-}2}$alkyl; and n is 0.

- 120 -

7.   A compound of claim 1 selected from
the group consisting of:

1-(3-dimethylaminophenyl)-2-methyl-5-phenyl-1H-
pyrrole-3-carboxylic acid ethyl ester;

1-(3-chlorophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-
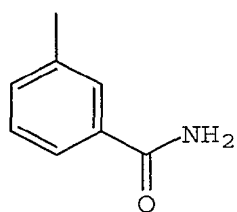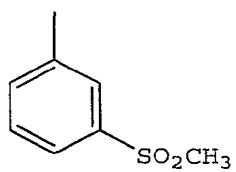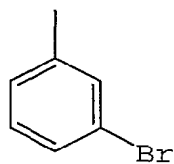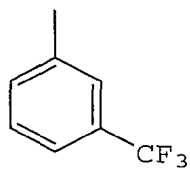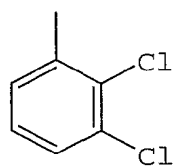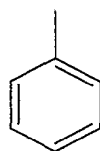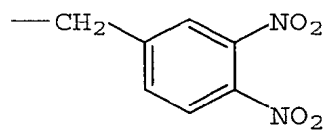carboxylic acid ethyl ester;

1-(3-chlorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

5-methyl-2-phenyl-1-m-tolyl-1H-pyrrole-3-carboxylic
acid ethyl ester;

2-methyl-5-phenyl-1-m-tolyl-1H-pyrrole-3-carboxylic
acid ethyl ester;

1-(3-bromophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

1-(3-acetylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

1-(3-acetylphenyl)-2-methyl-5-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

1-(3-methanesulfonylphenyl)-5-methyl-2-phenyl-1H-
pyrrole-3-carboxylic acid ethyl ester;

1-(3-methanesulfonylphenyl)-2-methyl-5-phenyl-1H-
pyrrole-3-carboxylic acid ethyl ester;

1-(3-carbamoylphenyl)-2-methyl-5-phenyl-1H-pyrrole-
3-carboxylic acid ethyl ester;

1-(3-carbamoylphenyl)-5-methyl-2-phenyl-1H-pyrrole-
3-carboxylic acid ethyl ester;

1-(3-dimethylaminophenyl)-5-methyl-2-phenyl-1H-
pyrrole-3-carboxylic acid ethyl ester;

1-(3-iodophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

2-methyl-5-phenyl-1-(3-sulfophenyl)-1H-pyrrole-3-
carboxylic acid ethyl ester;

- 121 -

5-methyl-2-phenyl-1-(3-sulfophenyl)-1H-pyrrole-3-
carboxylic acid ethyl ester;

1-(3-fluorophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

1-(3-fluorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

2-methyl-5-phenyl-1-pyridin-3-yl-1H-pyrrole-3-
carboxylic acid ethyl ester;

1-cyclohexyl-2-methyl-5-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

5-methyl-1,2-diphenyl-1H-pyrrole-3-carboxylic acid
ethyl ester;

2-methyl-1-(3-nitrophenyl)-5-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

5-methyl-1-(5-nitropyridin-2-yl)-2-phenyl-1H-
pyrrole-3-carboxylic acid ethyl ester;

1-(3-carboxyphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

1-(3-methoxycarbonylphenyl)-5-methyl-2-phenyl-1H-
pyrrole-3-carboxylic acid ethyl ester;

1-(3-bromophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

1-(3-iodophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

2-methyl-5-phenyl-1-(3-trifluoromethylphenyl)-1H-
pyrrole-3-carboxylic acid ethyl ester;

5-methyl-2-phenyl-1-(3-trifluoromethylphenyl)-1H-
pyrrole-3-carboxylic acid ethyl ester;

2-methyl-1-(3-nitrobenzyl)-5-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

5-methyl-1-(3-nitrobenzyl)-2-phenyl-1H-pyrrole-3-
carboxylic acid ethyl ester;

5-methyl-2-phenyl-1-pyridin-3-yl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-1-(4-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-1-(2-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

2-methyl-1-(5-nitropyridin-2-yl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-1-(4-nitrophenyl)-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(4-aminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(4-aminophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-aminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-aminophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-1-(3-nitrophenyl)-2-phenyl-1H-pyrrole-3-carboxylic acid;

5-methyl-1,2-diphenyl-1H-pyrrole-3-carboxylic acid; and

1-(3-ethoxycarbonylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester.

- 123 -

8.   The compound of claim 1 selected from the group consisting of:

1-(3-chlorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-acetylphenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-carbamoylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-dimethylaminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-fluorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-cyclohexyl-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

5-methyl-1,2-diphenyl-1H-pyrrole-3-carboxylic acid ethyl ester; and

2-methyl-1-(3-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester.

- 124 -

9.   The compound of claim 1 selected from the group consisting of:

1-(3-chlorophenyl)-2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-carbamoylphenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester;

1-(3-dimethylaminophenyl)-5-methyl-2-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester; and

2-methyl-1-(3-nitrophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid ethyl ester.

10.   The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 0.5 μM to about 25 μM.

11.   The compound of claim 1 having a PBL/TNFα EC_{50} of about 1 μM to about 20 μM.

12.   The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 0.5 μM to about 25 μM, and a PBL/TNFα EC_{50} of about 1 μM to about 20 μM.

13.   The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 100 μM or less.

14.   The compound of claim 1 having an IC_{50} vs. human recombinant PDE4 of about 50 μM or less.

15.   The compound of claim 1 having a PBL/TNFα EC_{50} of about 500 μM or less.

- 125 -

16. The compound of claim 1 having a PBL/TNFα EC$_{50}$ of about 100 μM or less.

17. The compound of claim 1 having an IC$_{50}$ vs. human recombinant PDE4 of about 100 μM or less and a PBL/TNFα EC$_{50}$ of about 500 μM or less.

18. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier.

- 126 -

19.   A method of treating a mammal having a condition where inhibition of a cAMP-specific PDE is of therapeutic benefit, said method comprising administering to said mammal a therapeutically effective amount of a compound having a formula

or

wherein R^1 is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)$alkyl, NR^4R^5, $C(=O)NR^5R^6$, $C(=O)O$alkyl, CO_2H, $OC(=O)$alkyl, nitro, halo, alkylthio, SO_2(alkyl), SO_3H, and haloalkyl;

- 127 -

R^4 and R^5, independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3.

- 128 -

20.   A method of modulating cAMP levels in a mammal comprising administering to said mammal an effective amount of a compound of

or

wherein R^1 is selected from the group consisting of optionally substituted alkyl, cyclo-alkyl, aryl, heteroaryl, alkaryl, aralkyl, hetero-aralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, hetero-aryl, aryloxyalkyl, aralkoxyalkyl, C(=O)alkyl, NR^4R^5, C(=O)NR^5R^6, C(=O)Oalkyl, CO_2H, OC(=O)alkyl, nitro, halo, alkylthio, SO_2(alkyl), SO_3H, and haloalkyl;

R^4 and R^5, independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3.

- 129 -

21. A method of treating a mammal having a condition where inhibition of a cAMP-specific PDE is of a therapeutic benefit comprising administering to said mammal a therapeutically effective amount of a pharmaceutical composition comprising a compound having a formula
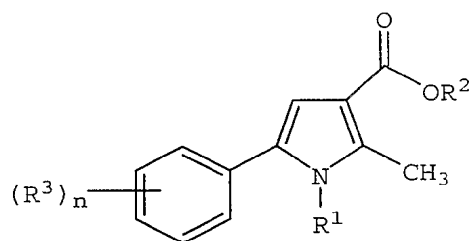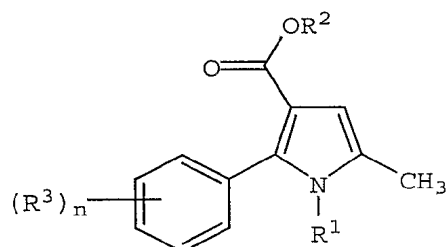
or

wherein R^1 is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, C(=O)alkyl, NR^4R^5, C(=O)NR^5R^6, C(=O)Oalkyl, CO_2H, OC(=O)alkyl, nitro, halo, alkylthio, SO_2(alkyl), SO_3H, and haloalkyl;
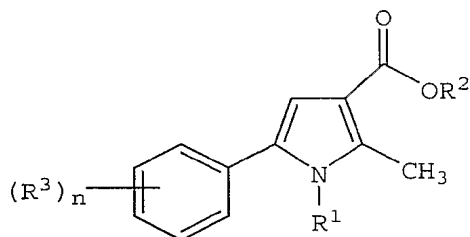
- 130 -

R^4 and R^5, independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and
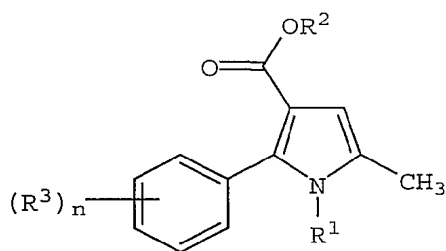
n is 0 through 3;

a pharmaceutically acceptable carrier; and,

optionally, a second anti-inflammatory therapeutic agent.

22.   The method of claim 21 wherein the condition is an allergic disease, an autoimmune disease, an inflammatory disease, an arthritic disease, or dermititis.

23.   The method of claim 21 wherein the condition is rheumatoid arthritis, osteoarthritis, gouty arthritis, or spondylitis.

24.   The method of claim 21 wherein the condition is thyroid-associated ophthalmopathy, Behcet disease, sepsis, septic shock, endotoxic shock, gram negative sepsis, gram positive sepsis, toxic shock syndrome, allergic conjunctivitis, vernal conjunctivitis, or eosinophilic granuloma.

25.   The method of claim 21 wherein the condition is asthma, chronic bronchitis, allergic rhinitis, adult respiratory distress syndrome, chronic pulmonary inflammatory disease, chronic obstructive pulmonary disease, silicosis, or pulmonary sarcoidosis.

- 131 -

26.   The method of claim 21 wherein the condition is reperfusion injury of the myocardium, brain or extremities as a brain or spinal cord injury due to minor trauma.

27.   The method of claim 21 wherein the condition is a fibrosis, keloid formation, or scar tissue formation.

28.   The method of claim 21 wherein the condition is systemic lupus erythematosus, a transplant rejection disorder, a graft vs. host reaction, or an allograft rejection.

29.   The method of claim 21 wherein the condition is chronic glomerulonephritis, an inflammatory bowel disease, Crohn's disease, or ulcerative colitis.

30.   The method of claim 21 wherein the condition is proliferative lymphocytic disease or a leukemia.

31.   The method of claim 21 wherein the condition is an inflammatory dermatosis, atopic dermatitis, psoriasis, or urticaria.

- 132 -

32.   The method of claim 21 wherein the
condition is a cardiomyopathy, congestive heart
failure, atherosclerosis, pyrexia, cachexia,
cachexia secondary to infection of malignancy,
cachexia secondary to acquired immune deficiency
syndrome, ARC, cerebral malaria, osteoporosis, a
bone resorption disease, fever and myalgias due to
infection, diabetes insipidus, a central nervous
system disorder, depression, multiinfarct dementia,
an anxiety or stress response, cerebral ischemia,
tardive dyskinesia, Parkinson disease, and
premenstrual syndrome.

33.   The method of claim 21 wherein the
mammal exhibits a minimal emetic response.

34.   The method of claim 21 wherein the
mammal is free of an emetic response.

35.   The method of claim 21 wherein the
mammal exhibits minimal adverse central nervous
system side effects.

36.   The method of claim 21 wherein the
mammal is free of adverse central nervous system
side effects.

37.   The method of claim 21 wherein the
second anti-inflammatory therapeutic agent is capa-
ble of targeting TNFα.

- 133 -

38. A method of reducing TNF levels in a
mammal comprising administering to said mammal a
therapeutically effective amount of a compound hav-
ing a formula

or

wherein R^1 is selected from the group
consisting of optionally substituted alkyl, cyclo-
alkyl, aryl, heteroaryl, alkaryl, aralkyl, hetero-
aralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of
alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, hetero-
aryl, aryloxyalkyl, aralkoxyalkyl, C(=O)alkyl, NR^4R^5,
C(=O)NR^5R^6, C(=O)Oalkyl, CO_2H, OC(=O)alkyl, nitro,
halo, alkylthio, SO_2(alkyl), SO_3H, and haloalkyl;

- 134 -

R^4 and R^5, independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3.

- 135 -

39. A method of suppressing inflammatory cell activation in a mammal comprising administering to said mammal a therapeutically effective amount of a compound having a formula

or

wherein R^1 is selected from the group consisting of optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, alkaryl, aralkyl, heteroaralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, heteroaryl, aryloxyalkyl, aralkoxyalkyl, $C(=O)$alkyl, NR^4R^5, $C(=O)NR^5R^6$, $C(=O)O$alkyl, CO_2H, $OC(=O)$alkyl, nitro, halo, alkylthio, SO_2(alkyl), SO_3H, and haloalkyl;

- 136 -

R^4 and R^5, independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and

n is 0 through 3

40. A method of inhibiting PDE4 function
in a mammal comprising administering to said mammal
a therapeutically effective amount of a compound

or

wherein R^1 is selected from the group
consisting of optionally substituted alkyl, cyclo-
alkyl, aryl, heteroaryl, alkaryl, aralkyl, hetero-
aralkyl, and heteroalkaryl;

R^2 is alkyl or hydrogen;

R^3 is selected from the group consisting of
alkyl, alkoxy, alkoxyalkyl, hydroxy, aryl, hetero-
aryl, aryloxyalkyl, aralkoxyalkyl, C(=O)alkyl, NR^4R^5,
C(=O)NR^5R^6, C(=O)Oalkyl, CO_2H, OC(=O)alkyl, nitro,
halo, alkylthio, SO_2(alkyl), SO_3H, and haloalkyl;

- 138 -
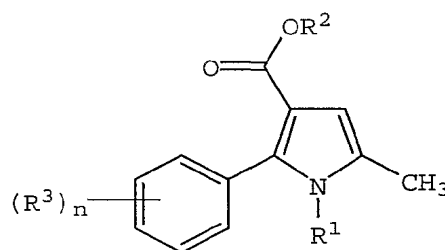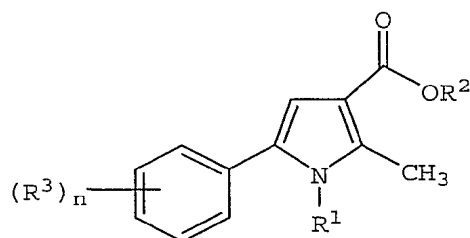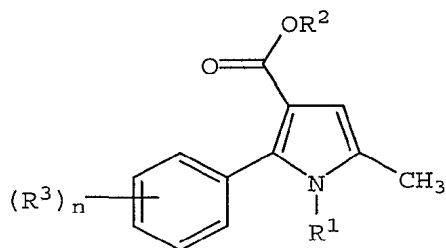
R^4 and R^5, independently, are hydrogen or alkyl, or R^5 or R^6 are taken together to form a 5- or 6-membered carbocyclic or heterocyclic ring; and
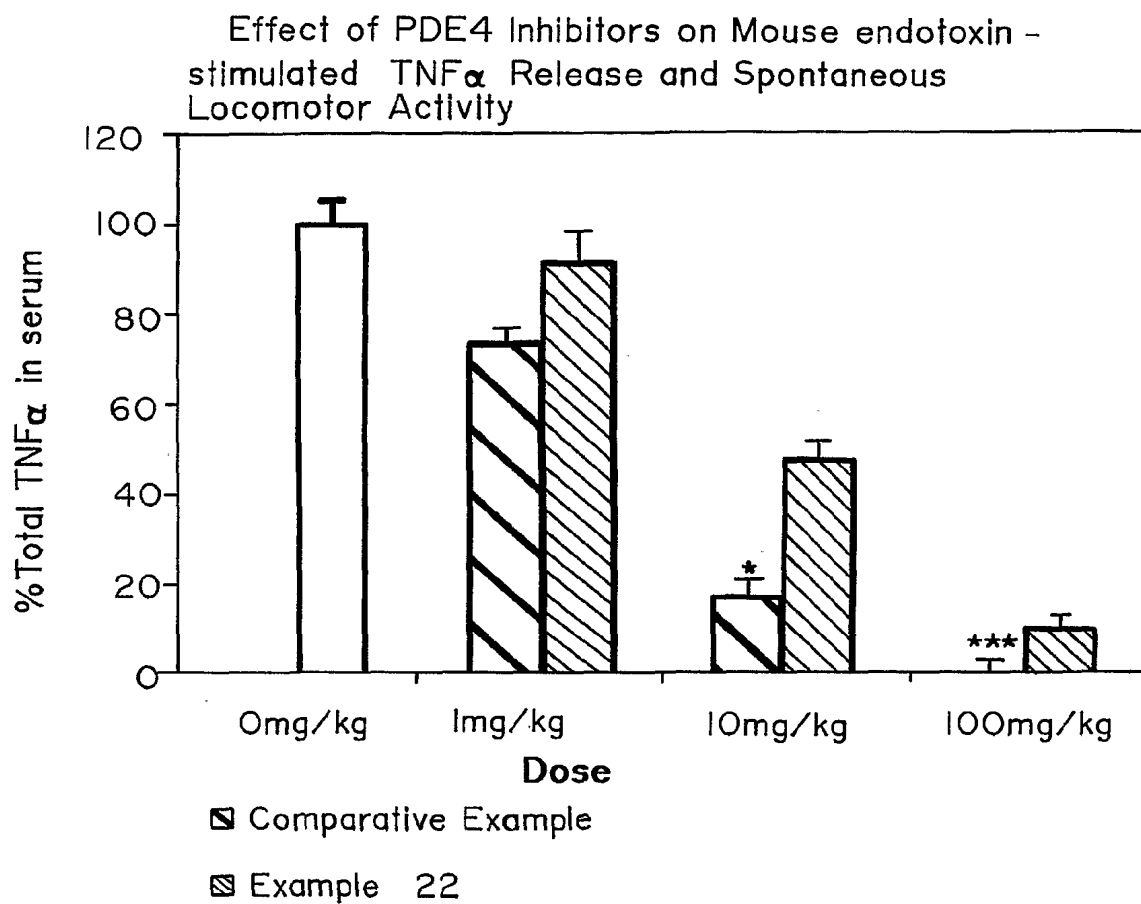
n is 0 through 3.

1 / 1

Effect of PDE4 Inhibitors on Mouse endotoxin – stimulated TNFα Release and Spontaneous Locomotor Activity

□ Comparative Example

▨ Example 22

FIG. 1

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7   C07D207/34   A61K31/40   C07D401/04   A61P29/00   A61K31/402

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7   C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 799 823 A (SANKYO CO., LTD., JAPAN) 8 October 1997 (1997-10-08) see formula (Ia-1), page 32 page 89, line 23 – line 54	1-5
X	CHEMICAL ABSTRACTS, vol. 97, no. 9, 30 August 1982 (1982-08-30) Columbus, Ohio, US; abstract no. 72206, GRINEV, A. N. ET AL: "Synthesis and biological activity of 4-oxoindeno'1,2-b!pyrrole derivative" XP002156580 abstract & KHIM.-FARM. ZH. (1982), 16(4), 433-8 ,	1-5

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| X | Further documents are listed in the continuation of box C. | X | Patent family members are listed in annex. |

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed , invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu- ments, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 January 2001	22 03. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Schmid, J-C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DEMIRAYAK, S. ET AL: "Synthesis of some 4-pyrrolylphenylthiazole derivatives and investigation of their antimicrobial activities" ACTA PHARM. TURC. (1997), 39(3), 133-136 , XP000945239 see compound 5d	1,2,5,6
X	KEATING, THOMAS A. ET AL: "Postcondensation Modifications of Ugi Four-Component Condensation Products: 1-Isocyanocyclohexene as a Convertible Isocyanide. Mechanism of Conversion, Synthesis of Diverse Structures, and Demonstration of Resin Capture" J. AM. CHEM. SOC. (1996), 118(11), 2574-83, XP002058962 see compounds 36 and 37 page 2578	1-3,5,6
X	PORRETTA, GIULIO CESARE ET AL: "Research antibacterial and antifungal agents. XII - Synthesis and antimicrobial activity of some Mannich bases of diarylpyrroles" FARMACO (1995), 50(9), 617-23, XP000945182 see compound 9 page 619	1-3,5
X	BACHECHI, FIORELLA ET AL: "A 1,5-diarylpyrrole derivative" ACTA CRYSTALLOGR., SECT. C: CRYST. STRUCT. COMMUN. (1995), C51(7), 1431- , XP000945267 see compound 2	1-3,5
X	ANTONUCCI, T. ET AL: "Characterization of the antiviral activity of highly substituted pyrroles a novel class of non-nucleoside HIV-1 reverse transcriptase inhibitor" ANTIVIRAL CHEM. CHEMOTHER. (1995), 6(2), 98-108 , XP000945183 see compound 46 page 101	1-5,18

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	ALMERICO, ANNA MARIA ET AL: "Polycondensed nitrogen heterocycles. Part 26. Aminopyrrolo'1,2- f!phenanthridines by decomposition and cyclization of 2-aryl-1-(3- azidophenyl)pyrroles" HETEROCYCLES (1994), 37(3), 1549-59 , XP000945175 page 1551 -page 1552	1-7
X	CERRETO, FELICE ET AL: "Synthesis and antimicrobial activity of some 1,5-diaryl-2-methyl-3- carbethoxy-4-(4-methylpiperazin-1-ylmethyl)pyrroles and some 1,5-diaryl-2-methyl-3,4-di(4-methylpiperaz in-1-ylmethyl)pyrroles" FARMACO (1993), 48(12), 1735-1746 , XP000945229 see formula 13 page 1738	1-6
X	ALMERICO, ANNA MARIA ET AL: "Polycondensed nitrogen heterocycles. Part 25. Aminopyrrolo'1,2-f!- phenanthridines by decomposition of 2-(3-azidophenyl)-1-arylpyrroles" J. HETEROCYCL. CHEM. (1994), 31(1), 193-8, XP000945177 the whole document	1-5
X	SCALZO, MARCELLO ET AL: "Studies on anti-Candida agents with a pyrrole moiety. Synthesis and microbiological activity of some '(1-alkyl),(1-aryl) and (1-benzyl)-5-aryl-3-carboxamido-2-methyl!p yrrole derivatives" FARMACO (1992), 47(7-8), 1047-53 , XP000945192 see formula 1 and compounds 15-23 page 1048 -page 1049	1-6
X	PORRETTA, GIULIO CESARE ET AL: "Chemotherapeutic agents with an imidazole moiety. III. Synthesis and microbiological activity of new 1,4-diarylimidazole and 1-(imidazol-4-yl)-4-(pyrrol-1-yl)benzenes" FARMACO (1991), 46(7-8), 913-24 , XP000945194 see compounds 6a-e page 915	1-3,5,18

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	COLOTTA, VITTORIA ET AL: "Tricyclic heteroaromatic systems. Synthesis of 1,3 and 1,2 disubstituted '1!benzopyrano'4,3-b!pyrrol-4-ones and structure-activity relationships a benzodiazepine receptor ligands" FARMACO (1991), 46(10), 1139-54 , XP000945195 see compounds 63-76 page 1148	1-5
X	TEXIER, F. ET AL: "Regioselectivity in 1,3-dipolar cycloaddition of munchnones with two electrophilic alkynes. Attempted rationalization" BULL. SOC. CHIM. FR. (1991), (NOV.-DEC.), 962-7 , XP000945368 see compound 6 page 963	1-6
X	PORRETTA, G. C. ET AL: "Research on substances with antibacterial and antifungal activities. Not VII. Synthesis and microbiological activity of new derivatives of 1,5-diarylpyrrole" FARMACO (1989), 44(1), 65-76 , XP000945349 see tabella (III) page 68	1-5
X	PORRETTA, G. C. ET AL: "Research on substances with antibacterial and antifungal activities. Note VI. Synthesis and microbiological activity of new derivatives of 1,5-diarylpyrrole and 1,4-pyrrolophenylene" FARMACO (1989), 44(1), 51-63 , XP000945348 the whole document	1-5
X	PETRUSO, S. ET AL: "Oxidative halogenation of substituted pyrroles with copper(II). Part II. Bromination of some ethyl 3-pyrrolecarboxylates and corresponding acids" J. HETEROCYCL. CHEM. (1990), 27(5), 1277-80 , XP000945179 see table 1	1-8

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	HE, MEIYU ET AL: "Cyclization of 1,2,3,5-polysubstituted pyrroles in electron impact" ORG. MASS SPECTROM. (1989), 24(4), 271-5 , XP000945280 the whole document	1-5
X	SCALZO, MARCELLO ET AL: "Synthesis and microbiological activity of new 1,5-diarylpyrroles" EUR. J. MED. CHEM. (1988), 23(6), 587-91 , XP000945383 the whole document	1-5
X	SCALZO, M. ET AL: "Compounds with antibacterial and antifungal activity. Part IV. Synthesi and microbiological activity of new 1,5-diarylpyrrole derivatives" FARMACO, ED. SCI. (1988), 43(9), 665-76 , XP000945201 the whole document	1-8
X	SCALZO, M. ET AL: "Compounds with antibacterial and antifungal activity. Part V. Synthesis and microbiological activity of new 1,5-diarylpyrrole derivatives" FARMACO, ED. SCI. (1988), 43(9), 677-91 , XP000945203 the whole document	1-5
X	CHEMICAL ABSTRACTS, vol. 109, no. 11, 12 September 1988 (1988-09-12) Columbus, Ohio, US; abstract no. 92698, GRINEV, A. N. ET AL: "Intramolecular cyclization of 5-(o-carboxyphenyl)pyrroles" XP002156581 abstract & KHIM. GETEROTSIKL. SOEDIN. (1987), (10), 1333-5 ,	1-5
X	CIRRINCIONE, GIROLAMO ET AL: "Polycondensed nitrogen heterocycles. Part 20. Reactivity of diazotized 1-(2-aminophenyl)pyrroles. Pyrrolo'1,2-f!phenanthridines" HETEROCYCLES (1986), 24(12), 3403-10 , XP000945339 see compounds 2b, 6b	1-8

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	PORRETTA, G. C. ET AL: "Pyrrolnitrin analogs. XI. Synthesis and microbiological activity of new 1,4- and 1,5-diaryl-pyrroles" FARMACO, ED. SCI. (1985), 40(8), 589-607 , XP000945205 see formula XIV and XV page 592	1-5
X	CHEMICAL ABSTRACTS, vol. 102, no. 9, 4 March 1985 (1985-03-04) Columbus, Ohio, US; abstract no. 78005, HUA, WENTING: "Vicinal group effect in Knorr-Paal condensation reaction" XP002156582 abstract & HUAXUE TONGBAO (1984), (10), 31-4, 7 ,	1-5
X	FILACCHIONI, G. ET AL: "Pyrrolnitrin analogs. VIII. Synthesis and antibacterial activity of new 1,4- and 1,5-diarylpyrroles" FARMACO, ED. SCI. (1983), 38(9), 630-9 , XP000945207 the whole document	1-5
X	FILACCHIONI, G. ET AL: "Pyrrolnitrin analogs. VII. Ethyl 1,4- and 1,5-diaryl-2-methylpyrrole-3- carboxylates from phenacylarylamines and ethyl acetoacetate" FARMACO, ED. SCI. (1983), 38(9), 619-29 , XP000945206 the whole document	1-5
X	CHEMICAL ABSTRACTS, vol. 97, no. 21, 22 November 1982 (1982-11-22) Columbus, Ohio, US; abstract no. 182142, HUA, WENTING ET AL: "Synthesis of sterically crowded polysubstituted pyrroles. (II)" XP002156583 abstract & HUAXUE TONGBAO (1982), (5), 281-4, 314 ,	1-5

—/—

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATTOLO, GAETANO ET AL: "Polycondensed heterocycles. Part XI. 1,3-Disubstituted 2-methylpyrrolo'3,2-c!cinnoline and 2-acetyl-3-methylpyrrolo'1,2-c!benzo'1,2,3!triazine" HETEROCYCLES (1982), 19(4), 681-6 , XP000973101 see compound 1	1-5
X	FILACCHIONI, G. ET AL: "Pyrrolnitrin analogs. VI. Unusual pathway in the Knorr synthesis betwee N-(4-nitrophenacyl)dimethylanilines and ethyl acetoacetate" FARMACO, ED. SCI. (1979), 34(11), 987-1004, XP000945210 the whole document	1-5
X	CHEMICAL ABSTRACTS, vol. 88, no. 11, 13 March 1978 (1978-03-13) Columbus, Ohio, US; abstract no. 74256, KEJHA, J. ET AL: "Preparation and evaluation of the antiinflammatory effectiveness of substituted 4-aroylpyrrole-3-carboxylic acids and 5-phenyl-2-methyl-3-carboxypyrrole-4-aliphatic acids" XP002156584 abstract & CESK. FARM. (1977), 26(6), 220-6 ,	1-6
X	CHEMICAL ABSTRACTS, vol. 63, no. 10, 8 November 1965 (1965-11-08) Columbus, Ohio, US; abstract no. 13189b, MCKINNON, DAVID M: "Feist synthesis of pyrrole-3-carboxylic esters" XP002156585 abstract & CAN. J. CHEM. (1965)43(9), 2628-31,	1-6

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 63, no. 12, 6 December 1965 (1965-12-06) Columbus, Ohio, US; abstract no. 16289g, SCHILFFARTH, KARLCHRISTIAN ET AL.: "aryl-substituted 1,1'-bipyrryls and their dissociation into radicals" XP002156586 abstract & CHEM. BER. (1965), 98(10), 3124-32,	1-6
X	CHEMICAL ABSTRACTS, vol. 55, no. 22, 30 October 1961 (1961-10-30) Columbus, Ohio, US; abstract no. 22337, FATUTTA, SILVANA: "ethyl alpha-(p-phenylphenacyl) acetoacetate and related heterocyclic derivatives" XP002156587 abstract & ANN. CHIM. (1961), 51, 252-65,	1-5
A	US 5 665 754 A (FELDMAN PAUL LAWRENCE ET AL) 9 September 1997 (1997-09-09) cited in the application the whole document	1-40
A	WO 92 19594 A (SMITHKLINE BEECHAM CORP) 12 November 1992 (1992-11-12) the whole document	1-40

| Box I | Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet) |

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 19-40 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound.

2. [X] Claims Nos.: 1,5
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

| Box II | Observations where unity of invention is lacking (Continuation of item 2 of first sheet) |

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1,5

The initial phase of the search revealed a very large number of documents
relevant to the issue of novelty. So many documents were retrieved that
it is impossible to determine which parts of the claim(s) may be said to
define subject-matter for which protection might legitimately be sought
(Article 6 PCT). For these reasons, a meaningful search over the whole
breadth of the claim(s) is impossible. Consequently, the search has been
restricted to the compounds of claim 2

The applicant's attention is drawn to the fact that claims, or parts of
claims, relating to inventions in respect of which no international
search report has been established need not be the subject of an
international preliminary examination (Rule 66.1(e) PCT). The applicant
is advised that the EPO policy when acting as an International
Preliminary Examining Authority is normally not to carry out a
preliminary examination on matter which has not been searched. This is
the case irrespective of whether or not the claims are amended following
receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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			AU	1665397 A	09-10-1997
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